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SOME FACTORS AFFECTING ADRENAL INSUFFICIENCY IN THE RAT

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In 1856 the adrenal glands were not considered essential for life by Philipeaux because a high percentage of his rats survived removal of both glands. Since that time Boinet (1895), Lewis (1923), Rogoff and DeNecker (1925), Scott (1923) and others have also had very low death rates for adrenalectomized rats. The low mortality rate was explained by Weisel (1898) when he showed that many of his subjects had demonstrable accessory tissue. For many workers, this presence of accessories was regarded as rendering the rat unsuitable for the investigation of complete uncomplicated adrenal insufficiency.

On the other hand, a number of recent papers (Pencharz *et al.*, 1931; Gaunt, 1933; Firor and Grollman, 1933; Martin, 1932; and others) report a high percentage mortality in the rat following double adrenalectomy. A few of these imply that some of their animals die of adrenal insufficiency twenty-four hours after the removal of the normally located adrenal glands.

The experiments here reported were undertaken in an attempt to determine the factors responsible for the widely divergent findings for the adrenalectomized rat; especially since the criterion used (death) is so definite. In addition, the results of adrenalectomy upon body weight, the gonads and the oestrous cycle were observed.

MATERIAL AND METHODS. We used over 300 rats of four different strains, which for convenience will be labeled A, B, C and D.[1] We differentiated between true strain variations and those due to dissimilar labora-

[1] A = Long-Evans (hooded) brought from Calif. by Prof. P. E. Smith.
B = Wistar (white) received through the courtesy of Doctor Brandt, N. Y. State Psych. Hosp.
C = Sprague-Dawley (white) kindly sent by Doctor Martin, Univ. of Wisc.
D = Pure black-hooded—received through the courtesy of Doctor Haterius, N. Y. U.

tory care as follows. Series I is composed of 239 rats born and raised in our department animal quarters. Series II includes only the 67 animals adrenalectomized shortly after being received from outside sources B, C and D.

The animals received a diet of table scraps daily and fresh lettuce every other day. Biscuits made according to Steenbach's[2] formula were kept in the cages at all times. After adrenalectomy this standard diet was supplemented by the McCollum[3] lactation diet. The animals always recovered more rapidly when this was used, but we found that the McCollum diet alone was not satisfactory for the maintenance of our operated rats.[4]

Great care was taken to keep a constant temperature in the operating room and animal quarters. We can confirm Hartman's (1931, 1932) finding that sudden temperature changes are bad for the health of adrenalectomized animals.

At operation a rigid aseptic technique was employed. The operator wore a sterile gown, mask and cap. These were sterilized in an autoclave for twenty minutes under a steam pressure of eighteen pounds per square inch. Threads and needles were kept submerged in a mixture of formalin and alcohol. The operator's forearms and hands were scrubbed with soap and water, washed in aqueous mercuric cyanide (1 to 1000) and then in 70 per cent alcohol. The back of the animal was shaved and washed with 70 per cent alcohol just prior to operation.

The adrenalectomies were performed under ether anesthesia. The gland was approached by an incision at the angle of the last rib and the erector spinae muscle. After the exposure of the gland its pedicle was picked up with a small pair of curved forceps placed at a safe distance from the

[2] Modified Steenbach diet—

Yellow corn meal	37.5 per cent
Ground whole wheat	37.5 per cent
Linseed oil meal	16.0 per cent
Casein	5.0 per cent
Ground alfalfa	2.0 per cent
Bone ash	1.5 per cent
NaCl	0.5 per cent

[3] McCollum lactation diet—

Whole wheat	67.5 per cent
Casein	15.0 per cent
Whole milk powder	10.0 per cent
Butter	5.0 per cent
$CaCO_3$	1.5 per cent
NaCl	1.0 per cent

[4] We wish to state that the excellent living conditions in the department rat colony, which play an important part in our results, are due to a constant and careful supervision by Prof. Philip E. Smith and his associates.

gland. The gland and its pedicle together with the surrounding fat were then dissected free and removed. By this method there is no possibility of inadvertently injuring the capsule as the gland itself is never touched. However, as a check the capsules were examined under a binocular microscope immediately after removal. If there was any doubt as to the intactness of the gland the animal was discarded from the series. Both glands were removed at the same operation.

TABLE 1

Mortality sixty days after adrenalectomy

STRAIN	NUMBER OF ANIMALS	NUMBER THAT DIED	PER CENT MORTALITY	PER CENT SURVIVAL
Series I—Rats born and raised in our laboratory				
A	141	6	4.2	95.8
B	60	4	6.6	93.4
C	24	2	8.3	91.7
D	14	0	0.0	100.0
Totals.....	239	12	5.0	95.0
Series II—Rats received from outside sources				
A	None			
B	35	16	45.7	54.3
C	20	8	40.0	60.0
D	12	11*	91.6	8.4
Totals.....	67	35	52.2	47.8
Series III—Some results reported in recent literature				
A	62	62	100.0	0.0
B	180	158	88.0	12.0
C	121	96	80.1	19.9
D†	130	124	95.0	5.0
Totals.....	493	440	89.2	10.8

* The rat that lived was pregnant.

† Includes three other strains, but no distinction is made by the author.

A careful search for accessory cortical tissue was made at each autopsy, but in many cases visible accessory masses could not be found. Those found varied considerably in size, appearance and location. The largest weighed 7.3 mgm. and the smallest were just visible under the dissecting microscope. The identification of small amounts of corticoadrenal tissue from serial sections is sometimes difficult because highly active tissue does not have the accepted normal appearance (Zwemer, 1934.)

RESULTS. a. *Survival and body weight.* More than 90 per cent of the 239 rats born and raised in this laboratory survived complete bilateral adrenalectomy. Less than 10 per cent died regardless of the strain used (table 1, series I).

TABLE 2

Mortality after adrenalectomy

Series I—Rats of strain A grouped according to body weight and age

BODY WEIGHT	AGE	NUMBER OF ANIMALS	NUMBER THAT DIED	PER CENT MORTALITY	AVERAGE WEIGHT CHANGE 60 DAYS AFTER OPERATION
grams					*per cent*
50–200	18–180 days	80	1	0.8	+57
200–300	6– 12 months	40	3	7.5	+14
300–360	1– 2 years	21	2	9.5	−12
50–360		141	6	4.2	

TABLE 3

The effect of adrenalectomy on the oestrous cycle

STRAIN	NUMBER OF ANIMALS	NUMBER SHOWING DISTURBANCES OF CYCLES	PER CENT AFFECTED
Series I—Female rats raised in our laboratory			
A	71	3	4.2
B	35	1	2.8
C	14	0	0.0
D	12	0	0.0
Totals.....	132	4	3.0
Series II—Female rats received from outside sources			
B	12	7	58.3
C	10	3	30.0
D	10	10	100.0
Totals.....	32	20	62.5

The pre-operative life history assumes importance when we consider the results from 67 apparently healthy animals received from outside sources (table 1, series II). The operative technique and post-operative care were identical in the two series.

Old rats have a slightly higher percentage mortality than the younger ones. The growth and body weight of the former are also somewhat affected by adrenalectomy (table 2). Accessories are very likely re-

sponsible for the difference in age susceptibility of the rat and the cat. In the latter species kittens are more susceptible to adrenal insufficiency than adult cats.

b. *The effect of adrenalectomy on the oestrous cycle.* Less than five per cent of the female rats born and raised in our laboratory showed a disturbance in the oestrous cycle after adrenalectomy (table 3, series I). On the other hand, over sixty per cent of those operated upon as received from outside sources showed prolonged or inhibited cycles (table 3, series II).

There is some correlation between a high per cent disturbance of the cycle and the mortality rate for the strain (series II, tables 1 and 3). This may be due to some factor other than the removal of large amounts of the animal's adrenal tissues, since in a much larger series of rats (series I, tables 1 and 3) the same operation failed to produce similar effects.

The oestrous cycle as determined by vaginal smear is not always an adequate criterion of sexual function. For this reason 14 virgin adrenalectomized female rats in heat were placed with males for a period of 12 hours. Nine of these became pregnant upon their first exposure and gave birth to normal litters, raising their litters until weaning. Three more became pregnant during their second exposure to males and also raised normal litters. The remaining two failed to become pregnant.

All of ten adult adrenalectomized male rats were found to be fertile when mated with normal females. Other adrenalectomized males of this strain (series I, strain A) have been used for breeding over a period of many months and found to remain fertile. Sections of the testes of adrenalectomized rats after autopsy showed no abnormal changes of the germinal epithelium. This is in accord with the finding for the adrenalectomized cat, in which it has been shown that degeneration does not occur (Elliot, 1915; MacMahon and Zwemer, 1929).

Adrenalectomized females have given birth to perfectly normal litters after mating with adrenalectomized males.

Discussion. Any discussion of the variable results following bilateral adrenalectomy of the rat is dependent on the interpretation of the term "corticoadrenal insufficiency." (Insufficiency of medulliadrenal and other chromaffine tissue is apparently compatible with existence.)

The corticoadrenal hormone available to any animal is the sum of its stored hormone plus that which can be readily prepared by its gland cells.

The normal metabolic demands for the cortical hormone seem to be increased by a variety of factors. Some of these are: chance or operative infection, traumatized tissue, prolonged anesthesia, a general "run-down" condition of the animal or an inadequate diet. Additional factors might be injected toxins or metabolism stimulators such as the thyroid hormone.

Adrenal insufficiency becomes apparent when the need for corticoadrenal hormone is greater than the amount available. We can therefore

have degrees of adrenal insufficiency depending on the ratio between $\frac{\text{the hormone available}}{\text{the demand factors}}$. This can be expressed as follows:

$$\frac{\text{Full complement of adrenal tissue}}{\text{Normal needs}} = \text{excellent health and growth.}$$

$$\frac{\text{Large accessory}}{\text{Normal needs}} = \text{apparent health with susceptibility to sudden excessive demand.}$$

$$\frac{\text{Small accessory}}{\text{Normal needs}} = \text{chronic insufficiency.}$$

$$\frac{\text{Complete corticoadrenalectomy}}{\text{Normal needs}} = \text{uncomplicated complete insufficiency.}$$

In the above proportions the denominator is constant for the normal metabolic needs of the body. The ratios would of course be changed by adding one or more of the additional demand factors to the denominator. The numerator would also be increased if sufficient time elapsed to allow hypertrophy of the cortical remnant, or if cortin were injected into the animal.

As an example of the many possible conditions that may occur, let us suppose that after adrenalectomy a rat still retained a large accessory. If the animal which was in poor pre-operative condition, had been subjected to prolonged anesthesia and some traumatization of tissue, and also developed a slight infection at operation the demand factors would greatly overbalance the hormone available. This rat would die within a few days but could be kept alive by injection of additional hormone. A similar animal operated upon under better conditions might survive unless a chance infection, a sudden temperature change, an inadequate diet (e.g., low sodium intake) or an injected toxin increased the demand in proportion to the supply. Again adrenal insufficiency becomes apparent. Under optimum conditions of operation and care, no excessive demands occur. This allows sufficient time for the accessory to increase functionally so that the hormone available is greatly in excess of the normal demand. The operated rat is then no longer distinguishable from a normal control by either observation or test.

Scott (1923) has pointed out the importance of optimum laboratory conditions in determining the mortality rate of a strain of rats different from those we used. Different standards in the control of demand factors above normal body needs may well account for the difference in the mortality rate of the adrenalectomized animals listed in table 1, series I–III.

When the demand factors are minimized so that a low mortality rate

results, the degree and duration of adrenal insufficiency in the rat are probably too slight to be adequately studied.

On the other hand, if the demand factors are increased so that the mortality rate is high, one must deal with the effect of these factors on the body as a whole, in addition to their action in inducing adrenal insufficiency.

The latter condition seems to explain the occurrence of changes in the oestrous cycle. Although inhibition of the cycle has been reported as a constant sequel to adrenalectomy by some workers (Kitagawa, 1927; Wyman, 1928; Martin, 1932; Corey and Britton, 1934) only four of 132 adrenalectomized female rats raised in our laboratory showed disturbances of their oestrous cycles (table 3, series 1). The fertility of both males and females also was not greatly impaired by adrenalectomy. This confirms the earlier work of Lewis (1923), Del Castillo (1928) and Schiffer and Nice (1930).

In uncomplicated adrenal insufficiency in the cat or dog there is almost always a loss in body weight. The variability of this change in the rat may well be due to factors other than a mere absence of the normal amount of corticoadrenal hormone.

Because of the variable results obtained by adrenalectomy in rats, we question the advisability of directly comparing results so obtained with those from other species with complete insufficiency, since one must always estimate the degree of insufficiency present in each individual rat.

SUMMARY

In 239 rats of four different strains the mortality rate after adrenalectomy was less than ten per cent. The same strains have been reported by others as having an 80 to 100 per cent mortality rate.

Operations upon an additional 67 animals and a careful study of the literature yielded some clues as to the reasons for the discrepancy. These are discussed in arriving at a definition of the term "corticoadrenal insufficiency."

Changes in the gonads and in oestrous cycles after adrenalectomy in the rat may be due to adrenal insufficiency, but seem to be induced by factors other than the removal of both adrenal glands.

Loss of body weight is indicative of adrenal insufficiency, but does not necessarily occur after adrenalectomy of the rat.

The results obtained with the adrenalectomized rat are not always comparable with those obtained in complete uncomplicated adrenal insufficiency.

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EFFECTS OF ADRENALIN INJECTION IN MODERATE WORK[1]

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The injection of adrenalin is followed by numerous and interdependent modifications of function. Of the 27 such "effects" listed by Peters and Van Slyke (1) perhaps the one which has elicited most argument is the influence of adrenalin on carbohydrate metabolism. Blood sugar and R.Q. increase and this was at one time thought to mean an increase in carbohydrate utilization. But it was found that lactic acid and ketone bodies increase also. Each of these processes may artificially increase the R.Q. and the formation of ketone bodies implies a decreased utilization of carbohydrate. Thus the view now generally held is that adrenalin promotes glycogenolysis and may actually retard the utilization of carbohydrate. Peters and Van Slyke (1) conclude that it is a direct antagonist of insulin because it attacks the same point in the carbohydrate cycle with the reverse effect of insulin.

These ideas have never been subjected to a rigorous test in exercise. While Ring (2) has reported that adrenalin reduces the rate of carbohydrate utilization in exercise, it was difficult to evaluate the magnitude of the effect partly because the subjects did not perform in a uniform manner from day to day. This variability in performance may have been related to the fact that the subjects were not in the fasting state.

We have carried out a series of experiments which seem to give a categorical answer to the question at issue: the influence of adrenalin injections on the utilization of carbohydrate in moderate exercise. Several other questions have been studied simultaneously and will be discussed in order, *viz.*, the blood sugar and lactic acid concentrations, the excretion of ketone bodies and the efficiency with which work is performed.

The protocol of one of 7 similar experiments on subject B. E. is given in table 1. Many details of experimental technique have been described elsewhere by Dill, Jones and Edwards (3). In their paper will be found information regarding the subject, the uniformity of his performance, the

[1] A preliminary account of this work was presented at the annual meeting of the Federation of American Societies for Experimental Biology in New York, 1934, and the abstract appeared in the J. Biol. Chem., **105**: xx, 1934.

[2] Fellow of the Rockefeller Foundation.

level of metabolism in rest and work and the accuracy of values for respiratory quotient. The experiment there described and two of the same sort serve as controls; in three other experiments adrenalin has been used[3] and in a seventh, glucose was given by mouth. The success of such experiments depends to some extent on the uniformity of response of the subject; it will be seen that this subject exhibited in a high degree uniformity in state from day to day.

TABLE 1

Protocol of a 24-hour experiment on subject B. E.

Seven such experiments were carried out on this man,—three controls, three with adrenalin and one with glucose.

TIME	
hrs.	
0.5	Rest
0.5–1.5	Resting metabolism; in this and in recovery periods the subject kept nose clip and mouth piece in place for 50–55 minutes while 4–6 portions of expired air were collected
1.5–3.5	Work metabolism at about 7 times the resting rate; in each such period expired air was collected 4 or 5 times, 10 minutes on each occasion
3.5–4.5	Recovery metabolism*
4.5–6.5	Work metabolism
6.5–7.5	Recovery metabolism
7.5–9.5	Work metabolism
9.5–10.5	Recovery metabolism
10.5–12.5	Rest
12.5–13.5	Rest metabolism
13.5–20.0	Sleep
20.0–21.0	Rest metabolism
21.0–23.0	Work metabolism
23.0–24.0	Recovery metabolism

* In calculating the mean R.Q. of recovery periods the value for the first 5 minutes was neglected. In the transition from work of this intensity to rest the CO_2 content of arterial blood shows little change but that of venous blood, and presumably of active tissues, decreases rapidly. Even though there is no lactic acid to be disposed of, the R.Q. in the first few minutes after moderate work is spurious.

The fuels used. During each 24-hour period and in one similar period without work, urine was collected and the total nitrogen excretion was determined. It will be seen from the values found in table 2 that the excretion of nitrogen is small and that the injection of adrenalin has little influence on it. Neither is it much influenced by this type of activity for the same subject excreted two-thirds as much nitrogen in 24 hours of

[3] The solution used contained 0.1 per cent adrenalin and was injected intramuscularly.

starvation without work as when 4,000 kgm. Cals. was expended in work during such a period. No more than 1.5 per cent of the energy production during work was obtained from protein assuming that the metabolism of protein proceeded at the uniform rate of 8.2 Cals. per hour in work and rest.

The non-protein respiratory quotients have been calculated both for work and rest and the results for the control experiments are shown in figure 1. Aside from some variability during rest and recovery, the values for R.Q. in each of the three experiments lie near a common curve. The fact that work and rest values are on the same curve means that in this

TABLE 2

Total nitrogen excretion

DATE	REMARKS	URINARY NITROGEN EXCRETION
		grams
Oct. 24 to 25	Control experiment	7.2
Nov. 21 to 22	Control experiment	8.1
Dec. 5 to 6	Control experiment	6.8
Mean		7.4
Nov. 7 to 8	Adrenalin, 3 cc.	7.3
Dec. 19 to 20	Adrenalin, 2 cc.	7.1
Jan. 9 to 10	Adrenalin, 1.5 cc.	6.3
April 12 to 13	Glucose, 400 grams*	5.4
May 21 to 22	Starvation without work, 24 hours	4.6

* Pure glucose in tablet form was ingested at the rate of about 2 grams per minute during the first and the third quarter-hour of each hour of work. Metabolism was measured in alternate quarter-hours. The subject was under the impression that fatigue was less at the end of the adrenalin experiments than after the control experiments. He was certain it was least after the glucose experiment.

individual a seven-fold increase in metabolic rate does not influence the proportion of energy derived from carbohydrate. This common curve seems to approach asymptotically a value slightly greater than 0.7. Certainly during the last two hours of work the R.Q. drops below 0.73. This implies the utilization of less than 8 per cent carbohydrate if the commonly accepted R.Q. of fat is correct. If it is 0.716 as reported by Cathcart and Cuthbertson[4] (4) then less than 5 per cent carbohydrate is being utilized. Certainly the energy production can be maintained at the rate of 500 Cals.

[4] They found that the R.Q. of abdominal fat is 0.711, of liver fat, 0.719, and of muscle fat, 0.717. The immediate sources of the fat used in muscular exercise are unknown but the mean value 0.716 has been used in our calculations.

per hour with a fuel consisting of more than 90 per cent fat. While such a fuel may require more oxygen and result in greater fatigue it appears to suffice for the essential chemical processes of moderate work for man as has been demonstrated for the dog by Anderson and Lusk (5).

The smooth course of the R.Q. throughout the control experiments provides an excellent basis for studying the effect of adrenalin injections.[5] The injections were not begun, however, until 30 minutes' work in the second period had been completed. It was thus possible to evaluate the initial state of the subject in each experiment.

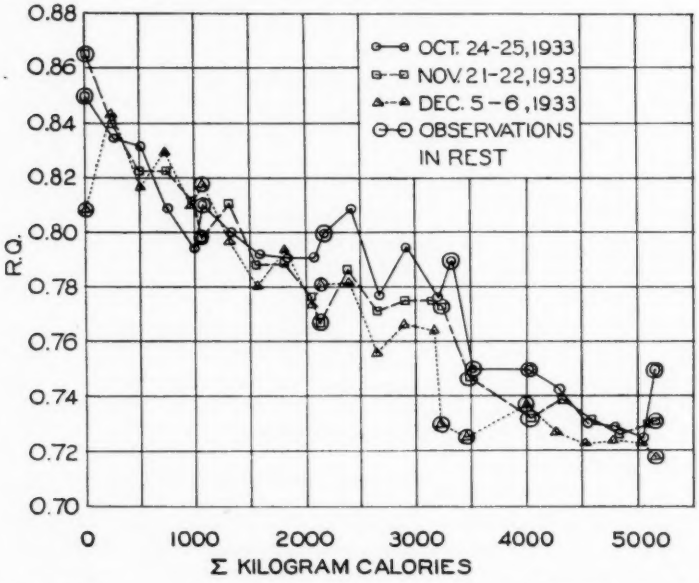

Fig. 1. R.Q. in control experiments. In the experiment of December 5 to 6 three injections of saline were made, the subject supposing adrenalin was employed.

The responses of the R.Q. to adrenalin injections are compared in figure 2 with the mean course of the three control experiments, shown by a heavy line. The values for R.Q. before adrenalin was injected lie near this line indicating that the subject began each experiment in the same initial state. The increases in R.Q. which occur after injecting adrenalin are so great that its effect cannot be questioned. The response after the injection

[5] In one of the control experiments, as noted in figure 1, 3 injections of saline were made, the subject supposing he was receiving adrenalin. There was no response in R.Q. nor in blood sugar. The subject observed that the injections did not seem to "pep him up" as had the injections of the previous experiment (when 1 cc. portions of adrenalin had been injected).

of ½ cc. is much less than when 1 cc. is administered. In a given experiment the response is very much greater during the second and third work periods than in the last period.

The question will at once arise as to the meaning of these changes in R.Q. Some lactic acid is produced and probably part of the early increase in R.Q. is due to excess evolution of carbon dioxide. The lactic acid thus formed must be removed eventually; as will be shown later, one hour after injecting adrenalin the lactic acid concentration has passed through its

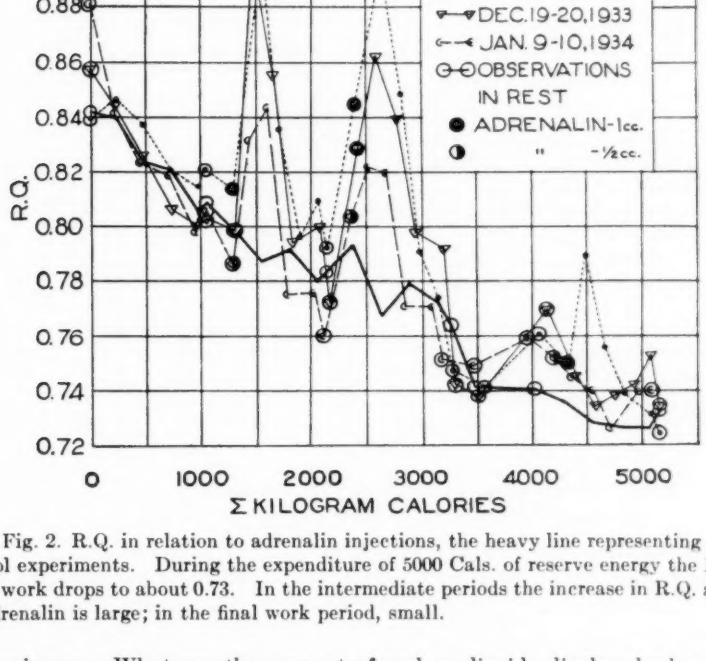

Fig. 2. R.Q. in relation to adrenalin injections, the heavy line representing control experiments. During the expenditure of 5000 Cals. of reserve energy the R.Q. of work drops to about 0.73. In the intermediate periods the increase in R.Q. after adrenalin is large; in the final work period, small.

maximum. Whatever the amount of carbon dioxide displaced when the concentration of lactic acid is increasing, an equal amount will be retained as the excess lactic acid is removed. Inspection of the curves shows that there is no tendency for the R.Q. to drop below the control curve, but that in each it merely returns to this curve. The actual course of carbohydrate utilization must differ somewhat from the R.Q. curve, rising and falling less abruptly, but the increased area under the R.Q. curve may be taken as a measure of increased carbohydrate utilization, so long as the metabolic rate is constant.

The quantitative aspect of this phenomenon is made more clear by table 3 in which are found the estimated amounts of carbohydrate utilized in each period. The mean increase during periods II and III, after injecting 1 cc. of adrenalin, was 67 per cent; after ½ cc. of adrenalin the corresponding increase was 18 per cent. While these averages cannot be considered precise there seems to be no question about the order of magnitude of the effect of adrenalin on utilization of carbohydrate in this grade of exercise.

TABLE 3

Influence of adrenalin on carbohydrate utilization

EXPERIMENT	CARBOHYDRATE USED				
	In 2 hours' work				Σ
	I	II	III	IV	
	grams	*grams*	*grams*	*grams*	*grams*
I. Control	87	70	68	21	246
III. Control	92	67	57	21	237
IV. Control	93	61	51	15	220
Mean of controls	91	66	58	19	234
II. Adrenalin	95	107*	107*	37*	346
Increment in carbohydrate used		41	49	18	
V. Adrenalin	88	98*	97*	32	315
Increment in carbohydrate used		32	39		
VI. Adrenalin	97	74†	72†	29†	262
Increment in carbohydrate used		8	14	10	
VII. 100 grams glucose each period	122	126	116	93	457

* Adrenalin intramuscularly, 1 cc. after ½ hour of work.

† Adrenalin intramuscularly, 0.5 cc. after ½ hour of work.

There are indications that adrenalin has a delayed effect, perhaps indirect in nature. Following the injection of 1 cc. in period II the R.Q. returned to normal at the end of work and remained near the control values during the rest period. However, the first observation in the succeeding work period was much higher than expected. Even in the fourth period after the overnight rest the same phenomenon is observed. It is particularly notable in the experiment of December 19 to 20. Two injections of adrenalin in periods II and III called forth the oxidation of 71 grams more carbohydrate than in the control experiments. No adrenalin was injected in period IV of this experiment, yet the R.Q. remained higher than in the control experiments. A further discussion of this phenomenon will be reserved for the present.

Some additional observations have been made on 5 other subjects working at the rate imposed on subject B. E. A single injection of adrenalin was made about 30 minutes after work started and observations were continued for 1.5 to 2.5 hours. Control experiments of the same duration were carried out on another occasion. In one subject 0.5 cc. of adrenalin had virtually no effect on R.Q. In the other 4 subjects the injection of 1 cc. had a fairly uniform effect, increasing the R.Q. to a maxi-

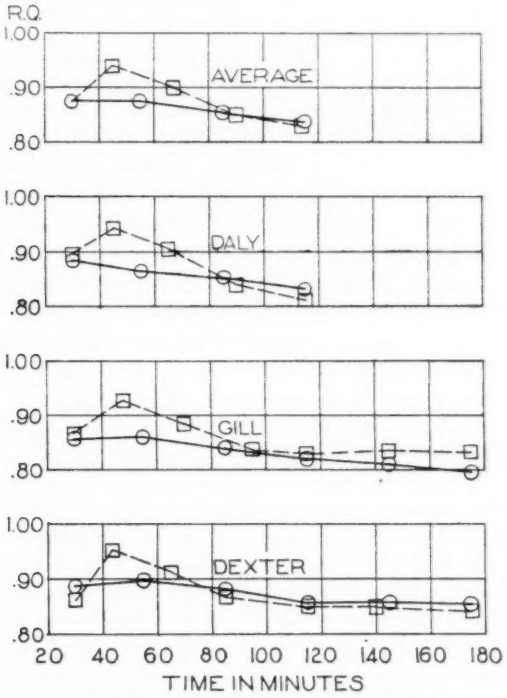

Fig. 3. The response of three subjects to adrenalin injections during work. The R.Q. was high when the injections were made; possibly on this account the increase in R.Q. is relatively smaller than in subject B. E.

mum about 30 minutes after the injection. In the case of one subject a direct comparison with the control experiment could not be made because the initial states were quite different.

The results on the other 3 subjects are shown in figure 3. The weighted mean values of R.Q. for 1.5 hours after adrenalin average 0.882 and for the corresponding period in the control experiments, 0.862. This implies an increase of 13 per cent in utilization of carbohydrate after adrenalin. This

result is qualitatively in harmony with the observations on B. E. although the increment in R.Q. is much less. The difference in response may be related to the relatively low initial R.Q. of subject B. E. For him this grade of work was easy and the R.Q. the same as in rest. For the others the work was nearer the limit of their capacity, the R.Q. was higher than in rest and the degree to which carbohydrate utilization could be increased was less. The best support for this explanation is the fact that when B. E. worked at a higher rate his R.Q. was increased above the level of rest and when 1 cc. of adrenalin was injected the subsequent increase in R.Q. was small.

Blood sugar and lactic acid changes. The course of the blood sugar curves in the 3 control experiments on subject B. E., presented in part elsewhere (3) (6), exhibited the same degree of uniformity as the values of the R.Q. The mean values are plotted as a function of caloric production in figure 4. The effect of adrenalin injections can be determined by comparison. We have previously found a lack of intimate relation between blood sugar and R.Q. (7) and these experiments furnish additional support for that finding. A comparison of figure 4 with figure 2 shows that whereas the second injection of adrenalin is followed by as great an increase in R.Q. as the first, the increase in blood sugar is not more than one-half as great. Furthermore, the injection of ½ cc. of adrenalin has an effect on blood sugar out of proportion to its effect on R.Q.

At various times in the experiments on B. E. venous blood has been obtained for determination of lactic acid. When adrenalin has not been administered lactic acid has varied between 10 and 20 mgm. per cent whether in work or rest. After injecting 1 cc. of adrenalin it rises to 30 or 35 mgm. per cent and then slowly returns to the rest level. The course of changes in blood sugar, blood lactic acid and R.Q. in one cycle in the experiment of December 19 to 20 is given in figure 5. Just as after the ingestion of glucose in exercise (7), the R.Q. passed through a maximum and returned to normal while the blood sugar was continuing to increase. The increase in lactic acid was much smaller than that of glucose, less than one-half as great expressed in milliequivalents. Although the concentration of lactic acid was still elevated after 90 minutes' work, it had returned nearly to normal by the end of the following rest period.

The excretion of acetone bodies. In the long experiments on B. E. the low level reached by the R.Q. corresponds to a ketogenic-antiketogenic ratio of about 2 and suggests the possibility of incomplete combustion of fat. Qualitative tests for acetone in urine by Rothera's test were negative in the early stage of the first work experiment and strongly positive at the end. The prospect was thus offered of additional evidence regarding the influence of adrenalin on carbohydrate metabolism—particularly so since Hubbard and Wright (8) have reported that the injection of 1 cc. of ad-

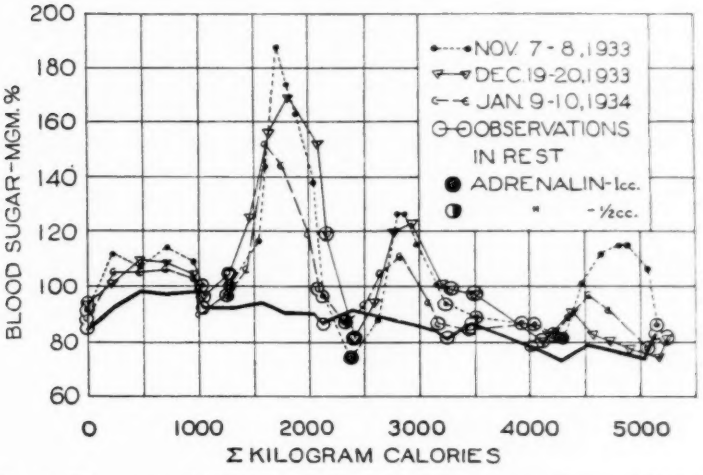

Fig. 4. The course of blood sugar curves during the expenditure of 5000 Cals. of reserve energy. The heavy line represents control experiments.

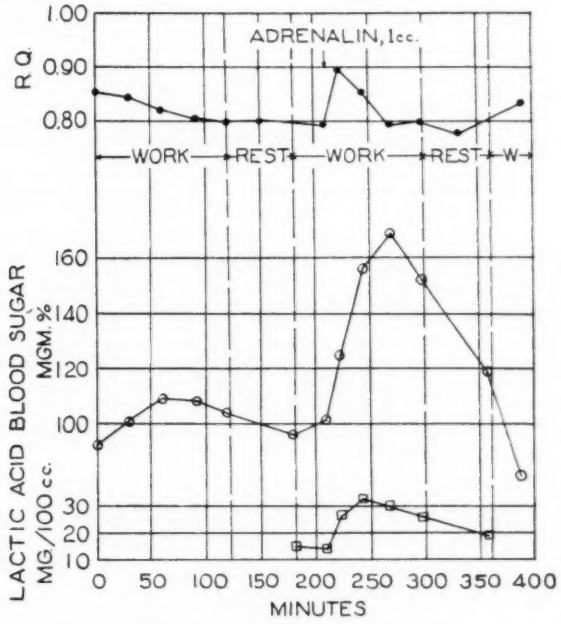

Fig. 5. Simultaneous observations on R.Q., lactic acid and blood sugar with relation to adrenalin injection in work.

renalin in rest is followed by an increase in ketone bodies of the blood. Their observation, in fact, is often quoted in support of the theory that the ability to oxidize carbohydrate is impaired by adrenalin. In all of our later experiments total urinary acetone bodies were determined quantitatively by the method of Van Slyke (9). The results are shown in table 4. In our experiments the injection of adrenalin did not increase the excretion of acetone bodies; on the other hand, the amount excreted was less in every case when adrenalin was used than the average in control experiments and the decrease was roughly proportional to the amount of adrenalin injected. In fact the output was as low with the injection of 3 cc. of adrenalin as with the ingestion of 400 grams of glucose.

TABLE 4

Total acetone bodies in urine

The values given are expressed as milligrams of acetone

EXPERIMENT	IN INITIAL REST	IN FIRST WORK	IN SECOND WORK	IN THIRD WORK	IN REST AND SLEEP	IN FOURTH WORK	IN 24 HOURS TOTAL
III. Control	0	6	4	25	187	33	255
IV. Control	0	5	4	40	333	69	451
II. 3 cc. adrenalin	0	13	19	24	61	23	140
V. 2 cc. adrenalin	0	12	19	23	90	45	188
VI. 1.5 cc. adrenalin	0	12	18	32	150	68	280
VII. 400 grams glucose	0	29	20	38	59	23	163
VIII. Starvation, no work	—	—	—	—	—	—	23

The amount of acetone bodies excreted is not wholly satisfactory as a measure of the amount formed for there may be some lag in the excretory process. However, there was in no case a considerable increase in acetone bodies in the blood. No direct determinations were made but the level of the CO_2 curve of blood was determined at intervals. This underwent only a slight decrease—on the average 1 or 2 m.-Eq. during a 24-hour experiment. This small quantity might be accounted for by the excretion of base in sweat and urine.

Lusk has stated that dogs and rats are very resistant to the development of ketogenic acidosis (10). Since we had obtained a rough measure of the effect of exercise plus starvation on production of acetone bodies in man an experiment was carried out to test the relative ability of the dog to oxidize fat completely. The performance of "Joe" has been described before. He is known to have two or three times the capacity of man for oxygen transport. The treadmill was operated at the same grade and twice the rate so that his rate of work output was twice as great as with our

human subjects. He began 24 hours after a meal, ran 6 hours on each of two consecutive days and 8 hours on the third. As compared with the 24-hour experiments on B. E., he accomplished in three days without food 5 times the energy expenditure at 2 times the rate. His urine remained virtually acetone-free by Rothera's test to the end, and he exhibited no great evidence of fatigue. Our observations on man do not conflict with the conclusion of Shaffer (11) that the threshold for ketonuria is reached when the R.Q. drops below 0.76. Actually it is not possible to draw a sharp line of demarkation in our experiments. We can say with certainty, however, that ketonuria results before the R.Q. reaches 0.73. In the dog evidently glycerol from fat is sufficient to complete the oxidation of fatty acids, even when the rate of fat oxidation is many times the rest level.

TABLE 5

Caloric production in work

EXPERIMENT	PERIOD I	PERIOD II	PERIOD III	PERIOD IV
I. Control	981	1007	1033	1025
III. Control	967	993	1016	1034
IV. Control	976	993	1025	1033
Mean of controls	975	998	1025	1031
II. Adrenalin	966	1011*	1020*	1032*
V. Adrenalin	975	1031*	1050*	1045
VI. Adrenalin	960	996†	1012†	1007†
VII. Glucose, 100 grams in each period	940	995	978	997

* Adrenalin intramuscularly, 1 cc. after ½ hour of work.

† Adrenalin intramuscularly, 0.5 cc. after ½ hour of work.

The effect of adrenalin on efficiency. This question may be dismissed very briefly: there is virtually no effect in these experiments. In the five cases in which 1 cc. of adrenalin was administered the heat production differed from the corresponding value in the controls by +13, −5, +1, +33 and +25, average +13 Cals. or 1.5 per cent. This means a decrease in efficiency of about 0.3 per cent. One explanation of the observed increase in heat production is that adrenalin has no effect on efficiency but that its calorigenic effect is about the same in work as in rest.

SUMMARY

About 5,000 Cals. of energy were expended by a fasting man in 24 hours. The energy derived from protein was about 1.5 per cent of the total. In the early stages of work about one-half the energy was derived from carbohydrate; in the last two hours, less than one-tenth.

In this subject the injection of adrenalin in three portions of 1 cc. each had no effect on protein metabolism, but increased the carbohydrate

utilization more than ½ over the corresponding period in the control experiments. Smaller quantities of adrenalin produced smaller results. In other subjects the injection of adrenalin had an effect smaller in magnitude but of the same sign. Further evidence that adrenalin, instead of impairing, facilitates the oxidation of carbohydrate in moderate activity is found in the fact that the excretion of acetone bodies was always less after adrenalin than in corresponding periods of control experiments.

The effects of adrenalin on blood sugar and lactic acid in work are like those in rest. When the concentration of blood sugar has reached its maximum, lactic acid has begun to decline while the R.Q. has already returned to the level of control experiments.

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BLOOD SUGAR REGULATION IN EXERCISE

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Recent investigations on this and related subjects have led to the following conclusions: *a*, with the cessation of work there may be an abrupt increase in blood sugar (1); *b*, blood sugar concentration during work is not a reliable index to the proportion of energy derived from carbohydrate (2); *c*, in football players before the game blood sugar is normal or nearly so; as the game proceeds, blood sugar concentration increases and may double in value (3); *d*, in hard work of long duration blood sugar may drop to one-half normal (4) (5). After having reached such a state the capacity for work may be increased by glucose administration (6).

In addition to these well-established facts Christensen (1) has reported that concentration of blood sugar tends to decrease during work, following a course fairly uniform but depending to some extent on the duration of work. It has been our observation that so long as exercise is continuous and reserves of carbohydrate ample, blood sugar is maintained near the resting level by increasing glycogenolysis to a rate commensurate with the rate of carbohydrate oxidation (2).

We have now extended our studies to work of a wide range of intensities and find that under some circumstances blood sugar increases in concentration while work is going on. In our subjects work which brings on exhaustion in from 10 to 40 minutes is usually associated with increases in blood sugar, which at the maximum average about 20 per cent, and in some cases exceed 40 per cent. The greatest rise observed was 66 per cent in a skilled runner experienced as a subject. On the other hand one of the smallest increases was in an equally skilled runner with no previous experience as a subject.

The experimental procedure has been described before (2). The subject came to the laboratory in the fasting state and rested for 30 minutes. After carrying out the work assignment on the treadmill he returned to the bed for a long recovery period. Blood samples were taken from the finger so that work could be carried on without interruption. The rate of energy production was measured from the oxygen consumption if a steady state could be attained. In other cases it was estimated from the treadmill rate

and inclination, assuming the same efficiency as in moderate work. We employed this inexact extrapolation method since only an approximate measure of work output was desired. The moderate work consisted of walking, the hard work of running and the maximum work of sprinting. In the last case the subject was compelled to support himself with his arms.

Two young men served as subjects for the principal experiments. One of them, B. E., is a trained distance runner referred to elsewhere (7). S. M. was the subject in 12 experiments with energy outputs ranging from

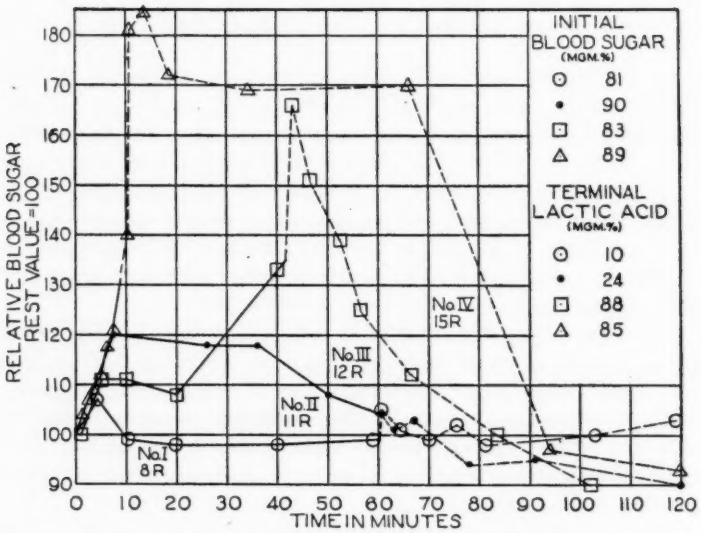

Fig. 1. Subject S. M. in work of moderate to intermediate intensity. Experiments I and II were terminated arbitrarily; the others ended with exhaustion. Solid lines indicate work; broken lines, recovery.

8R to 45R.[1] In two of these experiments the work was moderate and was terminated arbitrarily in 60 minutes; in 9 of the remaining 10 experiments work was carried on to exhaustion, the duration varying from 41 minutes to 23 seconds. So far as possible, conditions were kept uniform throughout the experimental period. The extent to which day-to-day variation in the state of the individual may modify the results has been considered and will be referred to below. In order to determine the variation in response of individuals a single experiment ending in exhaustion was carried out on each of several other subjects.

[1] The rate of energy output is expressed in terms of the rest level. Thus 45R means a rate of energy output 45 times as great as in rest, irrespective of the source of energy—aerobic or anaerobic.

The results of 4 experiments on S. M. in which the work rate varied from 8R to 15R are shown graphically in figure 1. The most moderate rate of work was associated with little change in blood sugar during the hour of work or during recovery. In the next grade of work blood sugar increased rapidly by about one-fifth, subsequently declining slowly until at the end of the hour of work it had returned to normal, becoming slightly subnormal in recovery. With a work rate of 12R the oxygen consumption was 3.2 liters per minute, nearly his maximal rate, and exhaustion resulted in 41 minutes. Blood sugar during work increased 30 per cent followed by a

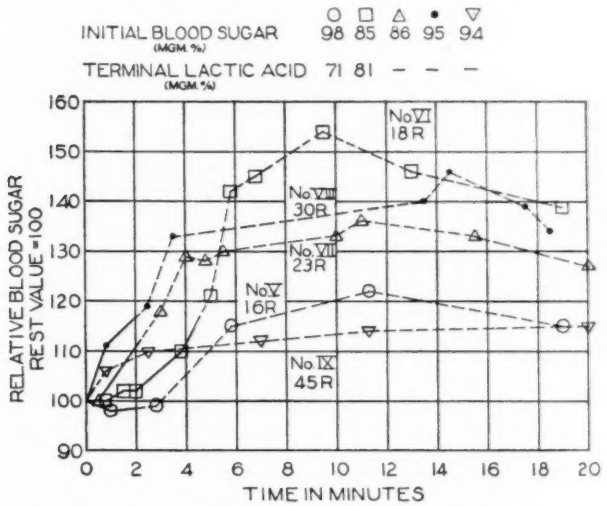

Fig. 2. Subject S. M. in work of moderate to maximal intensity. Experiment V was terminated arbitrarily; the others ended in exhaustion. Solid lines indicate work; broken lines, recovery.

further rise in recovery. At 15R some work was anaerobic and exhaustion occurred in 9 minutes with a maximal blood sugar during work 20 per cent above the rest level and 85 per cent above 5 minutes after the end of work.

The succeeding experiments on S. M. with further increases in rate of work, are shown in figure 2. It appears from the observations on this subject that the greatest rise in blood sugar during work is found in work carried on for many minutes near the limit of oxygen intake. As the rate of work approaches the maximum which can be carried on, even anaerobically, the rate of increase of blood-sugar concentration is no greater, and may be less, than that observed in less severe work of longer duration. In a short burst of intense exertion one finds little or no change in blood sugar; evidently there is not time enough for it to occur.

Experiments on subject B. E. will be referred to very briefly. Table 1 contains the results of 6 identical experiments on him, each 2 hours in duration and about 7R in intensity. In this moderate activity the blood sugar increased on the average 16 per cent. The results of 4 experiments in which the energy output ranged from 10R to 25R are shown in figure 3. The level of blood sugar reached at metabolic rates of 14R and 18R was higher than in any other subject studied. At 25R work was partly anaerobic; blood sugar increased 11 per cent during the 6 minutes of work and an additional 57 per cent during the first 7 minutes of recovery.

TABLE 1

Blood sugar during two hours' work

A blood sample was drawn after 30 minutes' rest and semi-hourly during uninterrupted work. The metabolic rate of the fasting subjects was increased about 7 times during work.

SUBJECT	DATE	BLOOD SUGAR, MGM. PER 100 CC.					MEAN CHANGES IN WORK
		Rest	$Work_{30}$	$Work_{60}$	$Work_{90}$	$Work_{120}$	
							per cent
B. E.	Oct. 24	75	89	93	95	96	+24
	Nov. 7	91	112	107	114	109	+19
	Nov. 21	85	88	99	96	101	+13
	Dec. 5	92	99	101	100	93	+7
	Dec. 19	92	101	109	108	104	+15
	Jan. 9	90	105	106	106	103	+17
Mean		88	99	103	103	102	+16
D. B. D.	Oct. 13	92	101	96	104	107	+11
Br.	Oct. 17	89	96	89		88	+2
Pa.	Oct. 19	122	102	99	104	89	−19
Dex.	Oct. 31	90	94	89	91	86	0
Gi.	Dec. 14	88	89	89	89	88	+1
Mean		96	96	92	97	92	−1

Day-to-day variations in the state of the individual seem to have no more than a minor influence on blood-sugar changes in exercise. Thus, the responses of B. E. in the 6 experiments of table 1 proved to be quite uniform. Subject S. M. exhibits greater variability. Figure 4 illustrates the results of four experiments, including no. IV of figure 1, in which the work intensity was 15R and the duration of work $9\frac{1}{6}$ minutes. Experiments X to XII were carried out on successive days about 3 months after no. IV. While the variation in blood-sugar curves is notable, it may be considered a second-order phenomenon as compared with the response of blood sugar to changes in work intensity.

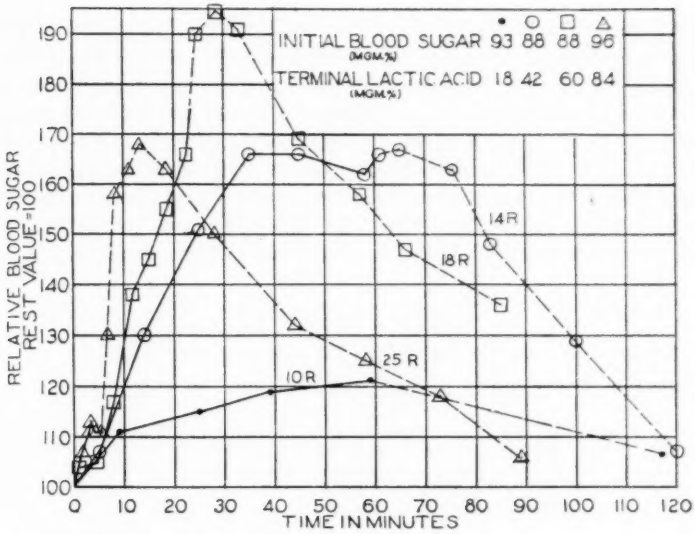

Fig. 3. Subject B. E. in work of intermediate intensity. Two experiments were terminated after 60 minutes arbitrarily; the others were ended in exhaustion. Solid lines indicate work; broken lines, recovery.

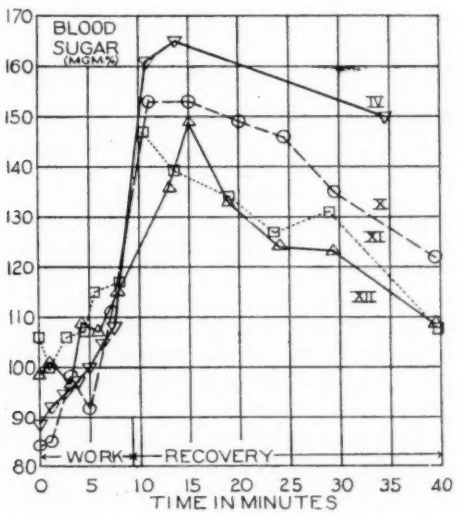

Fig. 4. Four experiments on S. M. with work of 15 R intensity carried on for 9.2 minutes. These results show a moderate uniformity of performance from day to day.

The question naturally arises as to whether these 2 subjects are representative in showing increases in blood sugar in activity which brings on exhaustion in from 10 to 40 minutes. Experiments carried out on 8 other subjects indicate that the response of S. M. is approximately average, and that of B. E. is greater than average. These 8 experiments are illustrated in figure 5. No subject failed to show a rise although in two cases it was terminal in nature and small in magnitude. The mean of the maximal blood sugar during work was 125, the rest value being 100. The individual range of the maximum was from 108 to 146. Such a variability

SUBJECT	W.C.	F.C.	C.D.	J.M.	G.C.	D.B.D.	D.B.D.JR.	J.K.
INITIAL BLOOD SUGAR (MGM.%)	89	108	93	101	87	96	91	101
FINAL LACTIC ACID (MGM.%)	70	48	58	65	72	48	21	72

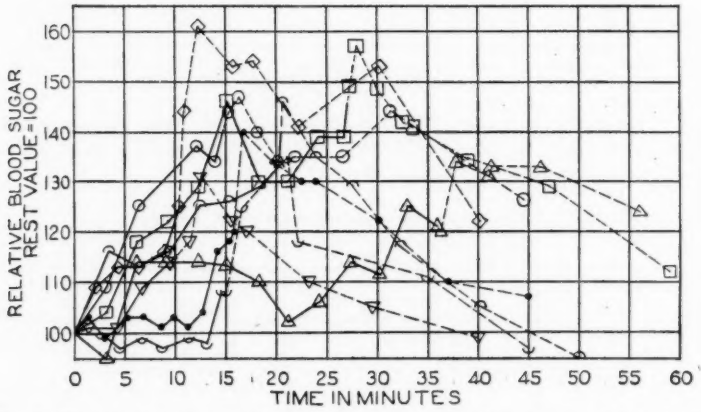

Fig. 5. Eight subjects in work to exhaustion. The duration varied from 10 to 36 minutes; all showed increases in concentration of blood sugar during work, the range being from 8 to 46 per cent. Solid lines indicate work; broken lines, recovery.

probably depends on variation in capacity for supplying oxygen and for oxidizing carbohydrate; it also implies varying capacities for mobilizing carbohydrate. There was no simple relation between degree of response and the age or the experience as a subject.

We have noted that the hyperglycemia of B. E. in work of intermediate intensity is greater than that of any other subject studied. This is also true in moderate activity. This will be evident from the comparison made in table 1. Five other subjects carried out the same work assignment. Three had virtually the same concentration of blood sugar in work and rest. One subject showed an increase of 11 per cent in work and

a fifth subject who started with an unusually high resting value had normal values during work. On the basis of these results as well as much unpublished material on other subjects we conclude that in moderate activity without exhaustion blood sugar usually remains unchanged. Neither is there an increase in blood sugar during recovery from such exercise: this has been shown by Trimble and Maddock (8).

In the foregoing experiments the work has been continuous and it may be of some interest to describe the results of a single experiment of a discontinuous character. Subject B. E. is capable of running continuously

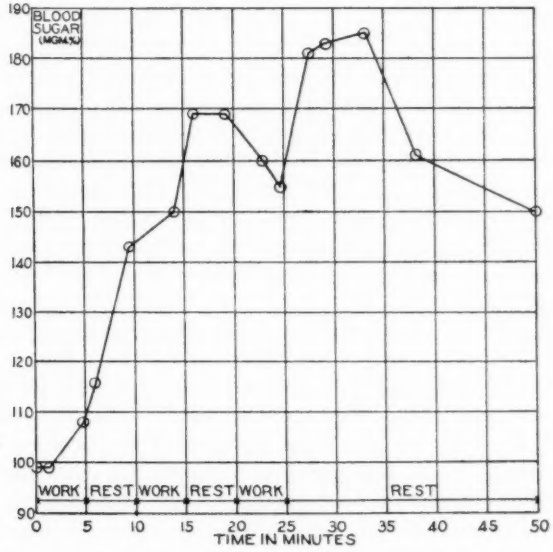

Fig. 6. Subject B. E. in discontinuous work of intermediate intensity. A high blood sugar level attained during recovery periods may be maintained during work.

6 or 8 minutes at the rate and grade chosen, 14.0 km. per hour and 7 per cent, respectively. In this case, work was arbitrarily limited to 3 periods of 5 minutes each with intervening rest periods of the same duration. It will be seen in figure 6 that the blood sugar increased throughout the first two work periods as well as in the first rest period and decreased in the third work period. The highest blood-sugar value, reached in the final recovery period, was approximately the same as after a continuous run of 6 minutes at the same rate (see fig. 3). However, it is evident that in such intermittent activity one may find a much higher level of blood sugar *during work* than when the work is continuous. This phenomenon may explain the high level of blood sugar observed in football players who during a

2-hour game may be active no more than 8 to 12 minutes. Whether the increases occur in work or in recovery, high blood sugar can persist through the short work periods of intermittent activity of an intense character.[2]

These observations on blood-sugar concentration in exercise and recovery provide a basis for speculation regarding the mechanisms involved. The facts presented can be considered conveniently in relation to three grades of exercise: 1, moderate, involving no oxygen debt; 2, severe, although chiefly aerobic; and 3, very severe, chiefly anaerobic.

1. *Blood sugar regulation in moderate activity entailing no increase in blood lactic acid.* In such activity the blood sugar shows little change in concentration either in transitional states or during the progress of work. This fact and particularly the constancy of lactic acid imply that *a*, the supply of oxygen is wholly adequate; *b*, adrenalin secretion is little increased, and *c*, little muscle glycogen is being used. The last two statements may be open to question, but they fit in with current ideas regarding carbohydrate metabolism (9). The rapid adaptation at the beginning and the end of moderate activity includes rapid responses in the rate of oxygen uptake, of carbohydrate oxidation and of the pulse. If glycogen is being furnished chiefly by the liver, it follows that there are rapid changes in glucose concentration in blood reaching the liver and in the circulation rate through the liver.

2. *Blood sugar in work which taxes the oxygen transport capacity.* In work of grade II one observes the highest blood sugar values, high lactic acid values and usually an overshooting of blood sugar concentration in recovery. Oxygen intake is maximal or nearly so and the R. Q. is increased (2), hence glucose oxidation is increased to a higher rate than in activity of grade I. However, glycogenolysis increases disproportionately and hyperglycemia during work results. There is no longer such labile control of the mechanism for glycogenolysis; its decrease in rate is less rapid than that of carbohydrate oxidation when exercise terminates. We are safe in assuming that glycogen is here being mobilized in both muscle and liver.

3. *Blood sugar regulation in work which is chiefly anaerobic.* The sprints and other short bursts of maximal exertion are representative of grade III

[2] On the three subjects studied by Christensen the changes in blood sugar during the transitional state after work were quite variable. He concluded that little could be learned about the concentration of blood sugar during work from the course of the recovery curve. However, the concentration of sugar a few seconds after work stops differs but little from its concentration just before stopping. Thus in 17 experiments on 13 subjects, including one dog, blood samples drawn from 0.2 to 0.7 minute after the end of work contained, on the average 5 per cent more sugar than samples drawn 0.2 to 1 minute before stopping. In 16 cases the differences ranged from −1 to +10 per cent and the 17th value was +15 per cent. If one took into account such other variables as the duration and intensity of work, an even closer uniformity might be established.

activity. Lactic acid concentration may not reach its maximum for some time after work stops, probably because of delay in diffusion, possibly in part due to delayed formation. After work of this sort the blood sugar does not reach the high levels observed after work of grade II. Indeed, in one case a run of 22 seconds to exhaustion was followed by only a slight rise in blood sugar.

A question that naturally arises is what rôle sympathetic stimulation of the adrenals plays in these three grades of activity. Cannon and Britton (10) have demonstrated in the cat that a minimal amount of muscular movement leads to acceleration of the denervated heart. We must, therefore, suppose that in all our experiments the sympathetic nervous system is called upon.

In the moderate activity of grade I, however, it is certain that adrenalin is not essential. It is likely that it is secreted sparingly with a precise and labile control. These statements are supported by the following facts: *a*, the heart rate returns to normal very rapidly after a few minutes of such activity; *b*, the blood sugar shows little or no overshooting when work terminates; and *c*, adrenalectomized animals are able to carry on ordinary activities with cortin only, as shown by Hartman, Brownell and Hartman (11).[3]

In work of grade II, which taxes the capacity for supplying oxygen, it is reasonable to suppose that adrenalin secretion is more copious and plays a major rôle increasing in effect as exhaustion approaches. There is possibly some dependence between blood sugar and lactic acid concentrations. It cannot, however, be an intimate relation. Thus, in intense work of very short duration, lactic acid may be increased as much as in the longer experiments of grade II and yet the increase in blood sugar may be negligible. Furthermore, we have observed considerable increases in blood sugar with but little change in lactic acid when work is carried on in a hot room to exhaustion. Here the emergency is no less real, but is of a different character and is associated with an increase in heart rate to its maximum (12). While it is tempting to suppose that the threshold for increased adrenalin secretion is the level of activity at which lactic acid begins to accumulate, the facts do not support a simple dependence between the two phenomena.

Work of grade II cannot be differentiated sharply from that of grade III. However, in work which is almost entirely anaerobic there is a very different reaction than in such typical activity of grade II as is illustrated in figure 5. There is not time enough in anaerobic work for the effects of adrenalin to become manifest. Consequently, there is little change in blood sugar during work and a moderate or slight increase in recovery.

[3] These authors state that *"Cortin treated adrenalectomized cats eat well, gain in weight, play, fight and act like normals."*

There are individual variations in capacity for supplying oxygen and presumably in capacity for mobilizing carbohydrate, hence considerable individual variation is to be expected in the level of blood sugar concentration maintained during work and recovery.

CONCLUSION

In easy work in which lactic acid does not accumulate, blood sugar remains near the rest level. If work brings on exhaustion in from 10 to 40 minutes blood sugar may be increased 10 to 66 per cent. Usually a still higher level is reached 5 to 10 minutes after the end of work. Activity which brings on exhaustion in less than 3 minutes with energy production chiefly anaerobic is characterized by little change in blood sugar during work and by moderate or small increases in recovery.

It is suggested that the following mechanisms are involved: in moderate work increased circulation rate and decreased glucose content of blood reaching the liver is accompanied by increased glycogenolysis in the liver. Thus there is no change in arterial blood sugar concentration either in work or in transitory states. While adrenalin may play a part it is not essential and if secreted the supply is not excessive. In severe work adrenalin secretion is abundant and the increase in glycogenolysis is greater than the increase in rate of glucose oxidation. Concentration of blood sugar increases considerably in work and still more when fuel consumption declines in recovery. Finally in anaerobic work the time is too short for the adrenals to come into effective action.

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RATES OF RESORPTION IN THE GALL BLADDER

Estimations Based on Experiments with Methylene Blue on Rabbits

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Former experiments (1) have shown that methylene blue appears in the bile of the rabbit within a few minutes after intravenous injection of the dye and that it reaches its highest concentration within one hour. The concentration then gradually drops to about 15 per cent of the maximum by the end of the sixth hour. The bile removed from the gall bladder at the same time, i.e., six hours after the injection, usually contains a concentration of methylene blue from two to twenty-two times that of the bile simultaneously obtained from the common bile duct. With this knowledge as a background experiments were planned in which bile was secreted against a constant pressure and was collected from the common bile duct during given intervals. From a knowledge of the volume and the methylene blue content of the collected bile and the amount of methylene blue in the gall bladder contents at the end of the experiment, it was possible on the basis of certain assumptions to calculate the mean rate of flow of bile through the cystic duct and also the amount of bile resorbed from the gall bladder per unit of time. The data thus obtained are here presented.

Method. With the animal under ether anesthesia the abdomen was opened and a glass cannula tied into the common bile duct close to its termination in the duodenum. The cannula was joined with a short rubber tube to the inlet *A* of a glass trap shown in figure 1. The trap was provided with a stopcock *S* and outlet tube *B* below, and a vent tube *P* near the top. The last was connected to a remote gas chamber with a hydrostatic pressure maintained precisely 50 mm. greater than that of the atmosphere. A double bubbling system *D* of tubes immersed in water to reduce the pressure of air from the laboratory blast line served efficiently for this purpose. The connections from the common bile duct to the trap were next freed from any air that might have retarded the flow of bile or by escaping later vitiate the volume measurements. After the proper adjustments were made the bile was carefully drained from the

trap and discarded. Collections were then commenced (over successive intervals of one-half hour), and 2 cc. of a 1 per cent solution of methylene blue per kilogram of body weight of the animal were injected into the marginal vein of the left ear. The volume of bile in each half-hour collection was measured and its methylene blue content determined by the method previously described (1). The experiments were concluded at the end of 2, 4 or 6 hours, at which time the entire content of the gall bladder was removed and its volume and methylene blue concentration determined.

The calculations were based on the following considerations:

It was assumed that in any given interval during the experiment the volume of bile which entered the cystic duct was approximately proportional to the volume which left the common bile duct, and also that the

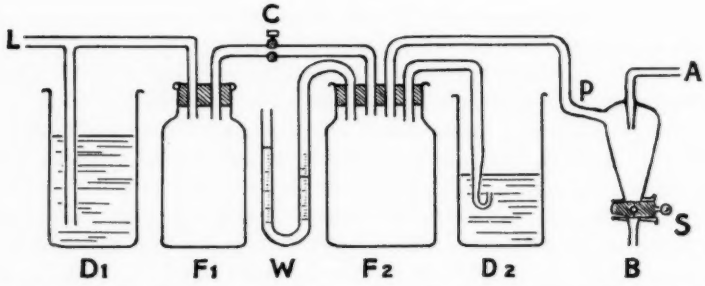

Fig. 1. L is the lead from the air line. F_1 and F_2 are gas pressure chambers associated with the bubbler-valves, D_1 and D_2. C is a Hoffman clamp; and W is a water manometer readable to 1 mm. The system is connected by a long rubber tube from F_2 to a vent, P, of the bile trap. The inside-sealed tube, A, is connected to the ductus choledochus.

amount of bile withdrawn from the common bile duct was replaced by an equal volume of bile of the same methylene blue concentration as that entering the cystic duct. Thus, as the experiment progressed, the ratio of total amounts of methylene blue in the collected samples and that which entered the gall bladder should be approximately the same as the ratio of the corresponding volumes. If v is the volume of bile which entered the gall bladder during the experiment and m the total amount of methylene blue which it contained and similarly V is the total volume of the samples and M their total methylene blue content, then $\frac{m}{M} = \frac{v}{V}$. Of these V and M were known, and m was approximated by the content of the gall bladder. Hence v could be estimated. Furthermore, if t is the time during which the samples were obtained (duration of collection), then the mean rate (r) of flow of bile through the cystic duct is $\frac{v}{t}$. Ob-

viously the mean rate of flow over a shorter interval (e.g., the first two hours) may be estimated similarly.

EXPERIMENTAL DATA. The essential data of each experiment are recorded in table 1. It may be seen that there is an individual variation in

TABLE 1

The transportation of bile and methylene blue by the biliary system of the rabbit after intravenous administration of the dye (2 ml. of a 1 per cent solution per kilogram of body weight)

RABBIT (MALES)		DURATION OF COLLECTIONS	VOLUME OF BILE OBTAINED			TOTAL METHYLENE BLUE IN BILE FROM		MEAN RATE OF FLOW OF BILE THROUGH THE CYSTIC DUCT		
			From D. choledochus		From gall bladder	D. choledochus	Gall bladder	In the time, t	In the first 2 hours	$\frac{r_2}{v_0}$
No.	Wt.	(t)	Total (V)	In first 2 hours (V_2)	(v_0)	(M)	(m)	(r)	(r_2)	
	kgm.	*hrs.*	*ml.*	*ml.*	*ml.*	*mgm.*	*mgm.*	*ml./hr.*	*ml./hr.*	*hr.*$^{-1}$
27	3.0	2	17.6	17.6	0.8	12.0	0.91	0.67	0.67	0.83
28	3.1	2	17.9	17.9	1.0	16.6	0.01	0.006	0.006	0.006
30	3.4	2	22.4	22.4	1.4	11.7	0.93	0.89	0.89	0.64
31	2.8	2	17.9	17.9	1.2	13.9	0.57	0.37	0.37	0.31
33	3.8	2	24.7	24.7	2.7	18.6	1.35	0.90	0.90	0.33
36	3.4	2	22.5	22.5	2.4	17.9	1.37	0.86	0.86	0.36
Mean*									0.74	0.49
a.d.									±0.17	±0.18
24	3.9	4	37.0	21.3	1.6	20.4	0.80	0.36	0.42	0.26
25	3.6	4	20.2	11.0	1.4	10.6	0.56	0.27	0.29	0.21
26	3.6	4	34.7	20.9	(0.9)?	19.4	(0.11)?	(0.05)?	(0.06)?	0.07
29	3.0	4	23.9	15.0	1.2	9.8	1.33	0.81	1.02	0.85
34	3.6	4	33.4	19.1	3.0	12.0	1.88	1.30	1.50	0.50
37	3.5	4	27.6	18.0	1.2	15.2	1.09	0.49	0.64	0.53
Mean*								0.65	0.77	0.47
a.d.								±0.33	±0.39	±0.19
32	2.9	6	28.5	13.6	1.2	13.7	1.20	0.42	0.60	0.50

* Rabbits 26 and 28 excluded.

the mean rate of flow $\left(\frac{V}{t}\right)$ of bile through the ductus choledochus and also in its pooled mean methylene blue concentration $\left(\frac{M}{V}\right)$. Similar individual variation is noted in the volume (v_0) and the methylene blue concentration $\left(\frac{m}{v_0}\right)$ of the contents of the gall bladder at the end of the experiment.

A decrease in the total output of bile was noted in the second and third two-hour periods of the experiments with a corresponding decrease in the estimated mean rate (r) of flow through the cystic duct.

If it is assumed that the amount of bile in the gall bladder was approximately the same at the beginning and at the end of the experiment, then the mean rate of flow (r) through the cystic duct must be approximately equal to the rate of resorption from the gall bladder. For the first two-hour periods the values of the relative rates of resorption $\left(\frac{r_2}{v_0}\right)$ have a mean of about 0.5 (hr.$^{-1}$). Thus it appears that under the experimental conditions given the gall bladder resorbed approximately half the volume of its contents per hour.

COMMENT. Substantial experimental evidence is available in favor of the view that under ordinary conditions, in the rabbit, bile does not leave the gall bladder through the cystic duct (1). The data here presented furnish a measure of the mean rate of flow of bile into the gall bladder and the amount of fluid resorbed from the viscus per unit time.[1]

SUMMARY

Methylene blue (2 cc. of a 1 per cent solution per kilogram of body weight) was given intravenously to rabbits and the bile secreted against a constant pressure was collected from the common bile duct in given intervals and the bile was removed from the gall bladder at the end of the experiment. The methylene blue content of each sample of bile was then determined. From a knowledge of the amount of methylene blue in the gall bladder contents and the volume and methylene blue content of the bile collected from the ductus choledochus, it was possible to estimate the amount of bile which entered the gall bladder during the experimental period. Furthermore, it was possible to estimate that under the experimental conditions given the gall bladder resorbed approximately half the volume of its contents per hour.

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[1] For the two-hour experiments essentially the same results are obtained from an alternative assumption that the rate of flow through the cystic duct is independent of the rate of flow through the ductus choledochus.

CHANGES IN NERVE-POTENTIALS PRODUCED BY RAPIDLY REPEATED STIMULI AND THEIR RELATION TO THE RESPONSIVENESS OF NERVE TO STIMULATION

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At times a nerve fiber is called upon to carry impulses at high frequency; for instance, Adrian, Cattell and Hoagland have shown that a jet of air directed against the skin of a frog may set up as many as 300 impulses per second in a single sensory fiber leaving the region. In a fiber functioning at such frequencies, changes occur in the potential signs of activity and in the level of excitability; but these have received little attention except for times considerably after the period of spike-production. The reason for this is not far to seek. Spikes and short after-potentials may be recorded with the aid of amplifiers having resistance-capacity coupling; but long after-potentials are grossly distorted. For the latter galvanometers may be used, but measurements made with them have a meaning only when the potential is changing very slowly and sufficient time has elapsed for the effect of the spike-potential on the galvanometer to have subsided. To record properly both spikes and after-potentials a system is needed which is at one and the same time adequate for potentials of both long and short duration.

METHOD. The conditions exacted by the problem are fulfilled theoretically by a non-reacting amplifier and the cathode ray oscillograph; but in practice certain difficulties arise which necessitate some compromises with respect to the several qualities in the interest of an optimum for all the qualities. For after-potentials an amplification of at least 100,000-fold is at times needed, even when the oscillograph (von Ardenne) is operated at a low anode voltage; and this amplification, to be serviceable, must be obtained with sufficient freedom from drift and without too great difficulty in the maintenance of the amplifier in balance. As a working arrangement an amplifier made of two pentode tubes (No. 57) proved serviceable. It gave an amplification of 40,000, which was sufficient for many purposes and was easily brought into balance by manipulation of the grid voltages within the permissible range. For additional amplification the output was coupled to the oscillograph through a third panel made with a 102-G tube.

Drift was occasioned much more by changes of the nerve potential than by the amplifier. Observations were seldom carried over one minute and no difficulty was encountered in producing the necessary stability for this length of time. One minute is more than ample to carry over to the point where after-potentials may be measured by a galvanometer unaided by amplification.

The after-potentials, which are very small, following as they do immediately after the spike, which is of a much higher potential, afford an especially exacting problem in amplification. In capacity-coupled amplifiers the filling of the series capacities by the amplified spike potentials causes the simulation of a long, low positive after-potential as soon as spike-production has ceased and makes the amplifier usable only for much shorter periods than would be the case if potentials of only one magnitude were involved. This effect is entirely absent in the battery-coupled amplifier, but a high potential followed by a low one still causes difficulty. The high resistance of the "57" tubes causes a delay to be introduced into the potential-change at the amplifier-output by any distributed capacity in the circuit, such as occurs in the batteries. For ordinary purposes delay is measured by the form in which a rectangular calibrating potential is returned by the amplifier (fig. 1 b); but such calibration methods are inadequate where after-potentials are involved. In the latter case the calibrating potential must equal in strength and duration the total area of all the spikes produced during a period of tetanization; and restoration of potential across the arms of the amplifier-bridges must be observed at the amplification necessary for the observation of after-potentials. Tests made in this way indicate that the delay is not of such a low degree that it can be disregarded. It is not ruinous in magnitude but in each case the detail under examination in the record must be examined with the proper calibra-

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Fig. 1. Calibration of battery-coupled amplifier with rectangular currents. Output taken from second "57" tube. *a*, 11 mv.; *b*, 0.7 mv.

Fig. 2. Test of calomel half-cells for polarization; 11 mv. applied to cells in parallel with input.

Fig. 4. Shape of shock delivered by the thyratron circuit with potentiometer output. 1.1 mf. condenser controlling thyratron.

Fig. 5. Tetani at different rates showing increasing height of spike-crests: *a*, 78 per second; *b*, 108; *c* and *d*, 230. *a*, *b*, and *c* on same time scale.

Fig. 6. Negative after-potential at a ceiling. Positive after-potential increases with duration of the tetanus. For reproduction two records have been printed superimposed.

Fig. 7. Negative after-potential. Two durations of stimulation of the same nerve. Frequency of stimulation 100 per second.

Fig. 8. Negative potential after a tetanus (*a*) compared with that after a single spike (*b*). The lines of zero potential have been drawn in.

Fig. 9. Short tetani at high frequency: *a*, 570 per second; *b*, 1000 per second.

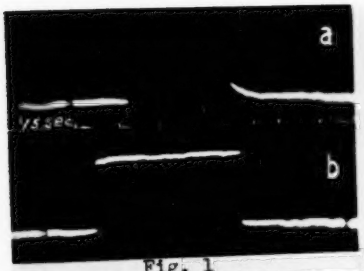
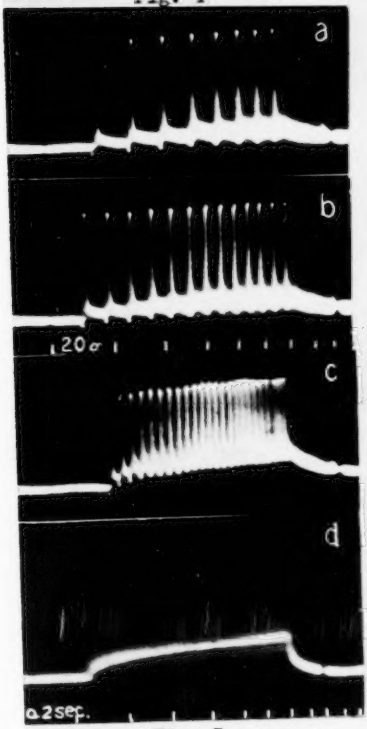


Fig. 1

Fig. 2

Fig. 4



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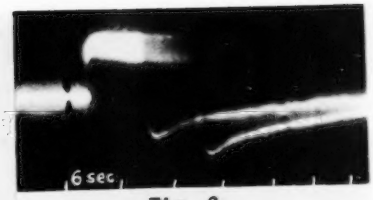

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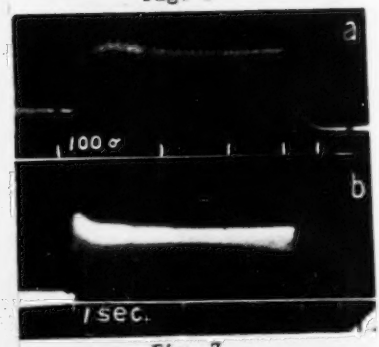

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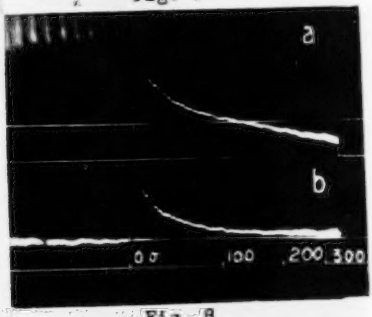

Fig. 8

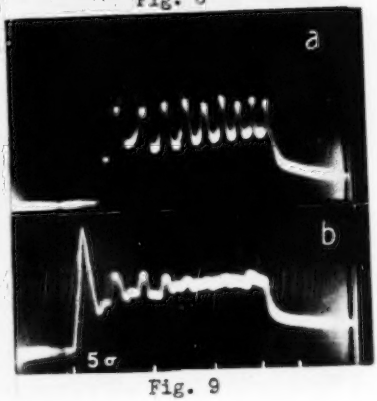

Fig. 9

tion curve in view. A calibration curve such as would apply to an average experiment is shown in figure 1 a.

Another feature of a battery-coupled amplifier which demands attention is the grid current of the first tube. Direct measurement of the latter showed it to have a magnitude of 8×10^{-9} amperes, too large to be considered free from the possibility of polarizing effects. As the grid current cannot be avoided when tubes of commercial hardness are used, and the nerve cannot be protected from it by means of a condenser, all phenomena observed had to be checked for the possibility of their being products of polarization. The check was made by comparing the potential picture returned from full input to the amplifier with that obtained when, by means of a potential divider, 5 per cent of the nerve's potential was applied to the input and the amplification increased twenty times. By this means the nerve could be observed with the current through it reduced to 10^{-10} amperes. Observations made in the two ways failed to show any obvious differences from each other. Differences, if there were any, would have to have been detected by a very careful analysis; therefore the system was considered usable.

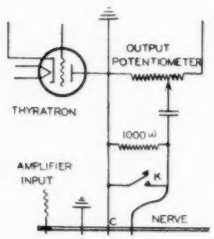

Fig. 3. Diagram of output of thyratron stimulator.

Calomel half-cells were used as leads. Stimulation was supplied from a thyratron oscillator of a form modified from that of Schmitt and Schmitt. Instead of using an induction coil an output assembly was constructed as in figure 3. With it the shock artifact was held down to a satisfactory size and the shocks were, in approximation, condenser discharges. Their duration could be controlled by the condenser in the thyratron circuit. The form of one, fifty times the length used for frog A fibers, is shown in figure 4. Application of the stimulation was controlled by means of a short-circuiting key, *K*, placed across the stimulating electrodes and mounted on the rotating contact-maker so as to be synchronizable with the sweep. Opening of the key, which was adjustable as to time, permitted a tetanus of any duration to be applied to the nerve. The frequency was controlled by a vacuum tube resistance in the thyratron circuit; and the number of shocks per second counted with the aid of the oscillograph.

Experiments in which no specific mention of the source of the nerve is made were performed on the sciatic nerve of the green frog.

A single response in any one nerve fiber results in a cycle of potential changes which have been described as the "spike," the "negative after-potential," and the "positive after-potential." All these potentials have different modes of behavior when a nerve goes into tetanic activity, there-

fore it will be convenient to describe them separately except in so far as their mutual dependence makes this impossible. To them must now be added a second positive potential not heretofore described.

The spikes. A succession of two spikes has often been described because of the technique of measurement of the refractory period. Much less has been said about a train of greater length. That staircasing occurs in nerve has been believed for some time, probably because of references in the older literature to the fact that in a tetanus produced by maximal shocks the negative variation increases with time. Davis and Brunswick state that the first of a series of nerve responses is smaller than those following; but, as they used a string galvanometer with a slack string, no differentiation of the part played by spike and after-potential was possible. In some unreported experiments made with the old standing-wave technique, in which the nerve was stimulated at a rapid rate, Erlanger and Gasser saw the spikes increase in height after the start of stimulation of fresh nerve, but no notice was taken of what the negative after-potential was doing at the same time. In order to clear up the matter some experiments were especially designed for the purpose.

The spike heights were investigated with shocks supermaximal for A fibers so that there could be no question of size-changes resulting from the number of units in operation. When the shocks were spaced at intervals longer than the relatively refractory period a characteristic result was an initial progressive increase of the absolute negativity of the spike crests with respect to the resting zero. The increase once started, several courses were open. After the first five to ten responses the crests in some instances settled down to a steady height; in others the increase continued for a longer period. In the case shown in figure 5 d the crest-level was still rising at the end of one second; and in other nerves the limit of the rise was not attained after several seconds. An alternative course was for the spike-height to rise to a maximum and then to decline to a steady level somewhat greater than the initial one. Progressive decline sometimes occurred but not in unpoisoned frog nerves stimulated at intervals longer than the relatively refractory period.

In spite of the increasing magnitude of the absolute negativity of the spike crests in a succession of responses, evidence was lacking of any increment of spike negativity when the latter was measured from the potential level residual from the preceding response. A search of the collection of records failed to bring to light any clear-cut examples in which the crest height could not be explained as a simple addition of the two potentials—the spike and the negative after-potential. Figure 5 (a, b, c and d) pictures a fair sample of the relationship. In general, except for decreases in the heights of the spikes above the base-line, the variation in the course of the absolute level reached by the spike crests depended upon variations in the behavior of the negative after-potential.

A word of explanation is necessary with respect to the height of the spike in relation to the supernormal phase of recovery. The latter is usually defined, after its mode of measurement, in terms of excitability (e.g., Adrian and Lucas, 1912; Gasser and Erlanger, 1930). Recently H. T. Graham has derived the additional information that velocity of conduction is also at a supernormal rate, but the spike height has been considered to be unchanged (Gasser and Erlanger, 1930; H. T. Graham, 1934). The reason for the latter conclusion is found in the method of testing used in supernormality experiments. Only two responses are involved. If the testing spike be measured from its own base-line, as is usually done, its height should be found to be unchanged. If measured in comparison with the conditioning spike the difference could easily escape detection even though supernormality were otherwise proved by the more sensitive method of thresholds. Only when the rise of height is progressive does the increase become obvious; and even as the matter now stands the spike considered as an isolated process cannot be considered to be supernormal.

When the frequency of stimulation is such that the shocks are spaced at intervals shorter than the relatively refractory period, the crest of the second response is, of course, lower than that of the first. As the responses now follow in succession the crests usually rise as in figure 5 (c, d) and may become higher than the initial spike itself. But if the rise of potential above the base-line be measured, it is found that actually the spikes are decreasing. Only by a rise of after-potential greater than the fall of the spike potential is an increase of the total potential made possible; and failure of it to occur results in a progressive fall of the crest level.

The negative after-potential. When a spike is set up during the relatively refractory period it rises to something less than the normal height. In contrast with this, if an after-potential be set up during the persistence of a preceding one but late enough to avoid complication by the spike potential, it rises to the previous maximum or somewhat higher, then continues (aside from the now increasing tendency to develop positive after-potential) along the same course that it would have followed if it occurred on an isolated response. The result, as shown by Zotterman and by Amberson and Downing with a ballistic method, is that two responses will not give twice the potential area of one unless they are sufficiently separated to permit the after-potential on the first one to be completed. In other words, the negative after-potential does not accumulate in proportion to the number of spikes; the uncompleted portion of the first potential is in effect omitted. Though not cumulative, there is still an increased tendency to after-potential production to an extent and duration which varies from nerve to nerve, as indicated in connection with the description of the spike crest-height. The increase of after-potential height may be continuous for some time, as in figure 5; it may stop when a steady state is reached, as in figure 6; or it

may continue to a maximum and decline to a steady state, as in figure 7. In addition, there should be mentioned the possibility of a progressive decline of the negative after-potential maxima, seen particularly in poisoned frog nerves and in mammalian nerves, which are not considered in this paper.

The steady state of the negative after-potential is of interest because, within limits, the longer the steady state is kept up the greater will be the subsequent positive after-potential (fig. 6). Manifestation of the tendency to go positive is apparent even during the tetanus. If the latter be stopped at any time the persistence of after-negativity is shorter than it would be on an isolated response. Figure 8 b shows the negative after-potential following a single spike. It was obtained by shortening the period of release from short-circuiting so that only one shock could get through. The period was then lengthened so that this shock became the last of a train, with the result that, although the negativity started from a higher level (fig. 8 a), it lasted less long because of the transition to the positive potential. When records are made in the form of figure 6 the increasing tendency toward positivity produces at any constant interval of stimulation a lower minimum of negativity just before each ensuing response; therefore, the maximum remaining the same, the band determined by the maximum and minimum becomes wider. Some nerves produce very little positive after-potential and then this widening of the band is absent.

In connection with the recording of potentials produced by repeated stimulation it should be pointed out that at stimulation intervals approaching the absolutely refractory period the base-line rises for other reasons than production of after-potential. Temporal dispersion of the spikes is increased by slowed conduction and increased latency (Blair and Erlanger), and the responses begin to alternate (fig. 9 a). Finally the responses become so random that the composite potential is no longer resolvable into its constituent parts but appears as a line at the level of about half the potential of a normal spike (fig. 9 b). An idea of the amount of overlap is gained by recalling the fact that a nerve fiber is absolutely refractory until the spike has nearly subsided.

The positive after-potential appears in the action potential of single responses immediately following the negative after-potential (Amberson and Downing, 1929; Gasser, 1933). When a nerve is tetanized the positive potential becomes larger and the period of negative after-potential becomes shorter. It was originally shown by E. Hering that the magnitude of the positive potential increases within limits with the duration of stimulation. This has been confirmed with the oscillograph; and as such records show that the peak of the positivity is of far too short a duration (e.g., fig. 12 c) to be recorded at its proper value with a slow galvanometer, a curve relating duration of stimulation to the maximum of positivity is reproduced in

figure 10. It represents the effect of stimulation of a nerve at 180 shocks per second, intervals of 15 minutes being allowed between the shorter tetani and 20 minutes or more between the longer ones. The size of the potential increases rapidly at first, then approaches a maximum. Analogously, if the duration be kept constant and the frequency varied, the positive after-potential again increases with the frequency, though at a decreasing rate. Figure 11 shows the values at the indicated frequencies for a tetanus having a duration of 2.5 seconds.

The durations of the positive after-potential after a tetanus were examined in a few nerves with the aid of a d'Arsonval galvanometer. They were found to last from 10 to 15 minutes—which duration is in confirmation of those found by Gerard (1929).

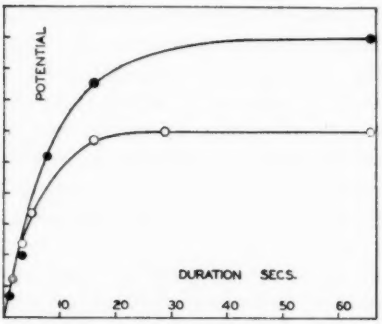

Fig. 10

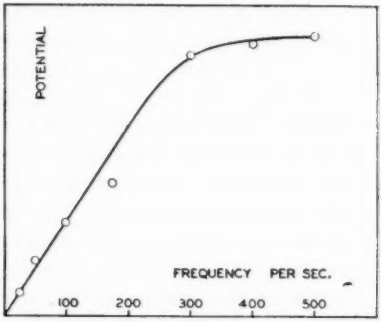

Fig. 11

Fig. 10. Size of the positive after-potential following tetani of varied durations. Two curves from the same nerve.

Fig. 11. Size of the positive after-potential following tetani of constant duration but varied frequency.

Excitability of nerve during the positive after-potential. In view of the supernormal excitability which obtains during the negative after-potential, it is not unexpected that excitability should be subnormal during the positive after-potential. Subnormality, as a phenomenon, has recently been analyzed by H. T. Graham (1933) in the special case of nerve poisoned by yohimbine or other substances having a similar action on nerve. After a single response excitability runs through a cycle in which appear in succession: a refractory period, a supernormal period, and a subnormal period. The third, or subnormal period, is not known to exist in unpoisoned nerve but may do so to an extent which has so far escaped detection, since some after-positivity is demonstrable in the normal cycle and the subnormal period in yohimbinized nerve is related to a greatly augmented positive after-potential (Graham and Gasser). In tetanized unpoisoned nerve

after-positivity is no longer small and subnormality should easily be detected.

The determination of threshold changes (for which the rapidly running spike is the test object) over the long period of the after-potentials presented a special problem to oscillographic technique, which was solved as follows. The sweep of the line across the tube face was made to occur about once in 2 seconds. Synchronized with this was a single stimulus from a thyratron stimulator set at such a strength as to bring into activity a fraction of the alpha fibers. When stimulation was once started it was kept going; thus the thresholds were brought to a state "equilibrated" to a frequency of one per 2 seconds. Then further stimulation was supplied in the form of a tetanus through another pair of electrodes, placed at a distance from the testing electrodes so as to avoid complication of the testing threshold by the local effects of the tetanizing current. The testing electrodes were situated between the tetanizing electrodes and the leads and placed close enough to the latter to eliminate temporal dispersion as a factor producing any serious effect upon the spike height.

Tetanization produced a positive after-potential having the form shown in figure 12 c. Segments of this curve, swept out as described for the recording of the spikes, appeared as a succession of lines across the tube face, gradually rising from the point of maximum positivity (positivity is down in the records) to the equilibrated level. On each sweep occurred a response to the testing shock; thus an index to the momentary excitability was obtained with a minimum of disturbance to the recovery curve.

The basis of the method of testing excitability lies in the fact that alpha fibers have different thresholds; therefore, a given shock strength at any stage of subnormality will be farther below threshold for some fibers than for others. From this it follows that the number of fibers responding becomes an indicator of the course of the recovery curve, though not a measure of it.

Return of excitability could be observed throughout its course on the screen of the oscillograph, and sample responses recorded by opening the camera shutter during the appropriate sweeps. A record is shown in figure 12 a. The topmost line shows the response at the equilibrated level; the lowermost a stage of positivity in which the shock was subthreshold, and the middle line a stage in which part of the fibers were responding. Full-sized spikes were not obtained until the base-line reached the equilibrated position. After a longer tetanus applied to the same nerve (fig. 12 b) a much closer approximation of the base-line to its equilibrated position was necessary before any fibers responded. In fact, all the stages of recovery had to take place after the uppermost of the recorded sweeps belonging to the recovery series. It has been pointed out that the positive after-potential increases with the duration of stimulation but at a decreasing rate

so as finally to reach a maximum. Apparently, when the tetani are of such a duration that the after-potential no longer increases, a trace is left in the nerve which necessitates more perfect recovery before any fibers will respond. The nerve excitability cannot be considered to be controlled by the absolute amount of positive potential alone. With the technique described, however, the matter cannot be analyzed further, as the after-potentials recorded involved more fibers than did the testing spikes.

During the positive after-potential the nerve is subnormal only in terms of excitability, not in terms of ability to respond. If the stimulation strength be increased the response is evoked at full value. Vészi, in 1912, observed that the response to a "moderately" strong induction shock is reduced during the positive after-potential. The present experiments are in confirmation of his only on the basis that his shocks were weak enough to permit some fibers to drop below threshold. Whether or not the spikes are higher during the positive after-potential, as they are in anelectrotonus, with which the latter has analogies (Verzár, 1926), we have not examined; but a statement by Woronzow (1924) indicates that such may be the case.

Subnormality must not be considered as a state of impairment super-induced by excessive activity from which recovery is possible only if restoration processes run their natural course. While it in all probability is a result of these restoration processes, still in a few thousandths of a second the excitability can be shifted from subnormal to supernormal. It is only necessary to throw in an adequate shock and test the excitability during the period of the negative after-potential following the latter. Such a sequence is illustrated in figure 13. Part *b* shows the size of an equilibrated spike as described in the preceding paragraphs. In the subnormal period following a tetanus it had the size of the lower curve in part *a*. Just before the next sweep following the one shown a stimulus above threshold strength was interpolated. This caused the regular synchronized response (upper curve, fig. 13 a) to be not only much larger than the preceding one but larger than the control equilibrated spike.

Fig. 12. Excitability of nerve during the positive after-potential: *a*, after a short tetanus; *b*, after a longer one. The upper lines show the control spikes, those below them the responses during the positive after-potential. *c* shows temporal course of positive after-potential.

Fig. 13. Same nerve as in figure 12: *b*, control; *a*, lower curve during positive after-potential; upper curve after an interpolated tetanus.

Fig. 14. Conduction in subnormal period. Sciatic nerve of bullfrog. Conduction distance 48 mm. *a*, nerve rested; *b*, during a positive after-potential.

Fig. 15. Positive after-potential with two convexities.

Fig. 16. Comparison of positive after-potential in a single response with that after a tetanus; *a*, single response; *b*, after a tetanus of 260 m.sec.; *c*, after a tetanus of 16 seconds; this record shows the train of spikes followed by a positive after-potential with a well defined *P1*.

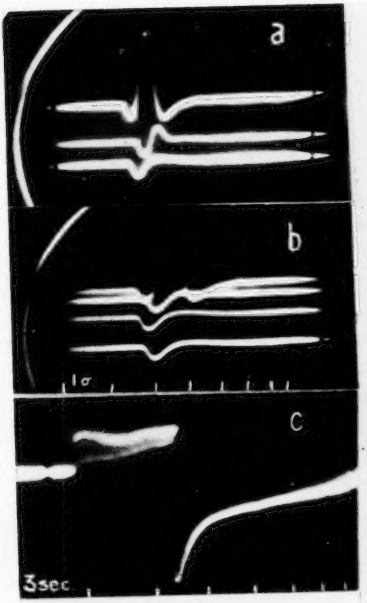

Fig. 12

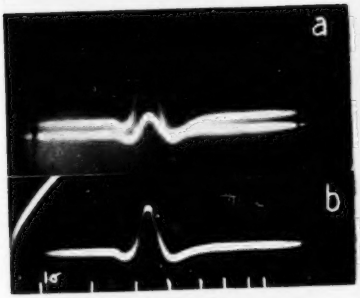

Fig. 13

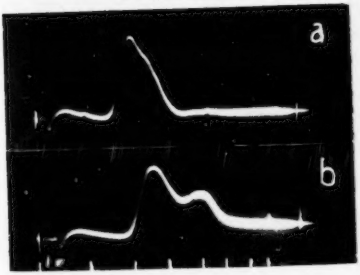

Fig. 14

Fig. 15

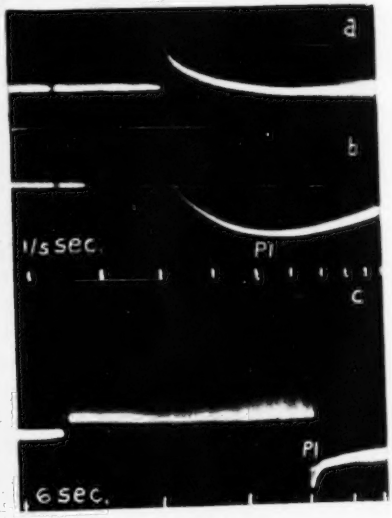

Fig. 16

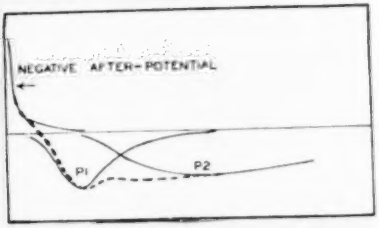

Fig. 17

Conduction in the subnormal period. During the negative after-potential H. T. Graham (1934) has shown a supernormal velocity of conduction. A symmetrical subnormal velocity is now shown during the positive after-potential. Experiments performed in the manner of those described in connection with figures 12 and 13, but with a longer distance of conduction and a faster sweep, always show the interval between the shock artifact and the start of the spike to be a few per cent longer in the subnormal period. Because of the uncertainty connected with the selection of the point marking the start of the spike these experiments can be considered only as qualitative and the actual measurements are therefore not tabulated. The differences at best are small and, in as much as the shock-artifact-spike-interval is made up not only of conduction time but of the latent period described by Blair and Erlanger as well, they might be due entirely to differences in latency. To settle this matter a few experiments were done with both leads on intact nerve and sufficient separation to permit discrimination of the start of the second phase. Any delay in this case in the passage from one electrode to the other must have been due to conduction velocity alone; and such a delay was found, amounting to a few per cent. Exact location of the start of the second phase again militated against precise data, but the greater temporal dispersion of the second phase gave good confirmation of the qualitative value of the measurements.

Slowed conduction during the subnormal phase and the resulting increase of temporal dispersion is strikingly illustrated in figure 14, made from records taken from the sciatic nerve of the bullfrog. The upper curve represents a response elicited after a period of rest; the lower curve a response during the subnormal period following a tetanus. In the latter the conduction time of all the fibers is increased. It shows for the fastest fibers in the time of the start of the wave and for the other fibers in the amount of temporal dispersion. Increase in conduction time by the same percentage amount in all fibers leads to increased dispersion, and it is not necessary to assume any differential effect. By cycles of rest and activity the action potential could be made to undergo repeatedly the change in form shown.

Form of the positive after-potential. The form of the positive after-potential curve is variable and in some frogs a positive potential cannot be obtained at all. In spite of the production of spikes without any abnormality of size or appearance, only a negative after-potential is obtainable. Instances of similar nature are found in Gerard's (1929) curves. When positivity appears in fully developed form it appears in two parts. Of these the first, in so far as it is visible, is of short duration, lasting about a second (figs. 6, 12, 15, 16). The second lasts to the end of the potential cycle. For descriptive purposes the two parts of the positive potential are designated for the sake of brevity as *P1* and *P2*. In some nerves *P2* is

the only potential visible. In others *P1*, while not appearing after mild tetanization, appears after tetani of high frequency or long duration; or it may be differentiated from *P2* if the temperature is lowered.

As *P1* is a phenomenon not previously observed, a search was immediately made for sources of artifacts which might produce it. So far none has been found. It cannot be a polarization effected by the tetanic stimulation as it is unaltered when the poles are reversed and absent when the nerve is narcotized. It is not an amplifier artifact, as shown by calibration curves made with rectangular currents, and it is not due to polarization of the leading-off electrodes. A rectangular current, impressed on the amplifier with the calomel half cells connected by a salt wick in parallel with the input, has the same form as in the absence of the cells (fig. 2). A more serious possibility is that it is a derivation from a second lead at the killed end. Several considerations speak against the latter possibility, however. To record positivity the change at the second lead would have to be negative. Near the killed end the negative after-potential is smaller and shorter than at the intact surface (Gasser, 1933); therefore it could not overpower the latter. Any negativity would have to be due to the spike process. Now it is to be expected that the effect of spikes at the injured end would be the same as in any deteriorated nerve, and in deteriorated nerve the spike gets smaller but not longer. Therefore it is difficult to associate any potential as long as *P1* with a spike process. Furthermore, when the nerve is made so monophasic by Bishop's cocaine method that no diphasicity of a single spike is visible even at the amplification necessary for after-potentials, *P1* is still fully developed (fig. 16 c). Thus all the evidence points to the conclusion that *P1* is a potential connected with some nerve process.

That *P1* and *P2* are separate and not parts of a single process producing positivity is to be inferred from their independent appearance, from the fact that the positivity as a whole appears at times with two convexities (fig. 15), and that it does not change as a unit when the temperature is altered. *P1* is increased in duration by cooling, Q_{10} being about 3. The effect of temperature on the duration of *P2* has not been studied. According to Garten the positive after-potential (which would be *P2*) is decreased in magnitude in cooled nerve, and this fact we have confirmed.

In order to ascertain what part of the potential cycle in a single response corresponds to *P1*, an experiment was performed in which the stimulation interval was shortened so as to permit only a single response to occur. Then the interval was lengthened in such a way that a response in the same position would be preceded by a train of responses. The nerve was characterized by a particularly large positive after-potential. In the single response (fig. 16 a) the latter reached a maximum of 15μv after 0.6 second. After a short tetanus the potential became larger and its maximum was reached earlier, at 0.4 second (fig. 16 b). When this augmented

wave was followed through to its full development after a longer tetanus (fig. 16 c) it was proven to be *P1*; therefore the positive after-potential seen after the single spike was shown to be in the position of the *P1* wave developed by a tetanus. After the tetanus the maximum value of the positive after-potential was 40 per cent of the spike-height.

Discussion. As the knowledge of the after-potentials develops it is becoming increasingly apparent that they are physiological phenomena and not laboratory artifacts. E. Hering, in 1884, insisted that the positive after-potential is a characteristic of fresh nerve and its disappearance is the first sign of fatigue. While his protocols show that his tetani were so close together that there must have been a positive potential persisting throughout the intervals between them, still there is no question that the after-potentials in deteriorated nerve are poor. This fact is readily shown by taking a lead near the killed end, in which case the positive after-potential—as is the negative—is smaller, measured in percentage of the spike height, than it is at a distance from the end. Nerves in general tend to have larger negative after-potentials compared with the positive as they become less normal.

After-potentials occur on all fibers, mammalian as well as frog; but there are quantitative differences which make generalization impossible. The positive potential is so marked on single responses of C fibers that, up to the recent elucidation of the subject by Bishop (1934), it has been regarded as the diphasic artifact of the spike. Negative after-potential, on the other hand, does not become obvious until these nerves are tetanized. Positive after-potential is also particularly prominent in fresh mammalian preparations. *P1* may there be followed by a negative potential and this in turn by a second much longer positivity, *P2*. The variations of the mammalian potentials will be described in another paper.

The after-potentials are nerve processes developing quite apart from the law of all-or-nothing. Their accumulation as a function of the total number of impulses the nerve has carried bespeaks a chemical origin; in fact, the positive after-potential is a phenomenon *within* the nerve, having a relationship to the number of nerve impulses producing it, roughly similar to that which humorally transmitted effects have to the number of impulses passing over autonomic nerve fibers (Rosenblueth; Gilson). As the after-potentials are accompanied by well defined states of irritability, which are absolute with respect to the normal level (H. T. Graham, 1934), and also are of long duration, their significance in connection with theories of activities in the central nervous system should be obvious.

One of the most interesting problems in connection with the cycle of action in nerve is why there should be so many potentials. The most promising mode of interpretation is to assume a chain of chemical reactions. Chains of this sort have been proposed by Gerard (1927) and they

can be elaborated to as great a degree of complexity as one pleases. The ionic species involved would not only be produced consecutively but would occur simultaneously; therefore any potentials which are signs of their existence would give the appearance of overlapping processes. Without going into detail it may also be pointed out that the variability of the potentials from frog to frog and with the degree of activity would have a plausible explanation.

From the form of the potential curves a suggestion, but not a definition, of the courses of the processes involved may be obtained. It may be said that attempts to derive the form of the total after-potential from a set of curves of logarithmic form, but with different rates of relaxation, such as would be possible if a set of catabolites existed awaiting disposal at various velocities, have met with no success. It seems necessary to suppose that each process must rise to a maximum, then decay, according to some such scheme as shown in figure 17. Variations in the size and duration of the constituents would account for all the variations encountered experimentally; for instance, the configuration of the positive potential is sometimes seen even though the net potential is negative. This could be accounted for if the negative constituent were higher and longer. The time of start of the positive potentials is intentionally left indefinite in figure 17.

SUMMARY

The potentials developing in frog nerve during and following a tetanus were recorded with a battery-coupled amplifier and a cathode ray oscillograph.

Spikes separated by more than their relatively refractory periods maintain a constant height above their base level. When more closely spaced they maintain the height of the second response or progressively decline.

The maximum of the negative after-potential tends to increase at first (staircasing). It approaches a steady state higher than the initial value, either directly or after passing through a maximum.

The spike potentials are added to the residual negative after-potentials giving the appearance of staircasing to their crests.

The positive after-potential is cumulative. Curves showing its increase with frequency and duration of stimulation are given. It may attain a maximum, equivalent to 40 per cent of the spike-height.

Excitability and conduction velocity are subnormal during the positive after-potential.

Subnormal excitability can promptly be changed to supernormal by the negative after-potential following a response to a stimulus above threshold.

The form of the positive after-potential indicates that it is dependent on two separate processes. The first one called *P1* has a shorter duration

than the second, *P2*. *P1* has a temperature coefficient of about three for ten degrees.

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THE RATE OF OXYGEN CONSUMPTION OF THE ISOLATED TERRAPIN HEART WHEN PERFUSED WITH VARIOUS SOLUTIONS

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Loeb (1900) in an effort to explain the inability of sodium chloride to maintain functional beats in cardiac tissue ascribed a "poisonous" effect to this salt. Howell (1901) questioned this poisonous effect except insofar as any other salt is likewise poisonous in being unable to maintain cardiac activity when used as the sole content of a perfusing medium. The observation of Warburg (1930) that certain tissues consumed far more oxygen when they were kept in a solution of sodium chloride alone than when kept in a Ringer's solution appears to lend some support to Loeb's theory concerning the action of sodium chloride on cardiac tissue. Since marked changes in the cation content of a solution of inorganic salts used as a medium for perfusing the heart are usually reflected in the behavior of that organ, it appeared to us worth while to study the oxygen consumption of the isolated heart when perfused with various modifications of a Ringer's solution and with an isotonic dextrose solution.

EXPERIMENTAL PROCEDURE. The method chosen consisted in recirculating a buffered, oxygenated solution through the heart for a definite period of time and determining the oxygen necessary to maintain complete saturation of the solution under constant conditions. The apparatus used was devised in this laboratory. It consisted essentially of two 50 cc. burettes standardized against each other, clamped firmly so that their graduations corresponded, and joined together at their lower ends with a "T" tube, the third arm of which was in connection with a short length of 17 mm. glass tubing which served as the heart chamber. The first of these burettes served as an oxygen chamber, the second, as a pressure regulating chamber. At the top, each had an outlet which could be closed or opened to the atmosphere. The oxygen chamber was in connection by a "T" tube with a 100 cc. round-bottom tube which served as a perfusion reservoir. This tube was inverted above the oxygen chamber. Below the heart chamber was a similar round-bottom tube which served as an overflow reservoir. Each of these tubes had an atmospheric outlet and was in

connection with the other tube through a 100 cc. hypodermic syringe which made recirculation of the fluid an easy matter. Between each unit of the system and its neighbor were interposed short lengths of pure gum tubing so that rate of flow could be governed through the use of screw clamps. The arm of the "T" tube entering the top of the oxygen chamber was drawn into a fine capillary tube from which was suspended a thermometer. The perfusing fluid thus enters the oxygen chamber in a fine stream, strikes the thermometer and trickles its length through an atmosphere of pure oxygen.

To operate this apparatus, the fluid selected for the experiment is saturated with oxygen at room temperature by bubbling oxygen freely through the solution for an hour. The entire system is then filled with the solution; the first burette is then filled with oxygen displacing the fluid therein through the second burette. The atmospheric connection of the first burette is then opened allowing fluid from the second burette to displace oxygen until the menisci in the two burettes are level; the first burette is then closed to the atmosphere, the second left open. The perfusion fluid is allowed to enter the heart through the right precaval vein, all other vessels being ligated, and permitted to escape through extensive incisions in the walls of both ventricles. The rate of flow is regulated to keep the two menisci level. Temperature changes were prevented within the fluid by the application of heat or cold to the overflow reservoir and within the gas by like applications to the oxygen chamber. At the conclusion of the period of experimentation, the volume of oxygen removed is read directly from the oxygen chamber.

The chemicals used in this study were Merck's and Baker's Analyzed. The solutions were buffered to maintain a pH of 7 to 7.2. The volume of oxygen was reduced to standard conditions and calculated in cc./gm. of tissue. The experimental time was 1.25 hour. At the conclusion of the experiment, all chambers of the heart were laid open, blotted dry, and the heart weighed. The composition of the Ringer's solution used was as follows:

	Per cent
Sodium chloride	0.650
Potassium chloride	0.014
Calcium chloride	0.012
Sodium bicarbonate	0.020
Sodium dihydrogen phosphate	0.001

Modifications were made by leaving out of this solution the ions indicated in the chart below; the dextrose solution was buffered with the bicarbonate and dihydrogen phosphate.

EXPERIMENTAL RESULTS. Averages of oxygen consumption and gross changes in rate for thirty-five isolated terrapin hearts perfused with various solutions are set forth in table 1.

When perfused for 1.25 hours there was little variation in the rate or amplitude of terrapin hearts on Ringer's solution; no change in rate but a gradual falling off in amplitude on sodium chloride; a gradual slowing with greater relaxation in diastole on sodium chloride plus potassium chloride; no change in rate but stronger beats on sodium chloride plus calcium chloride; at first much stronger and more rapid beats but later markedly weakened beats on dextrose alone.

DISCUSSION. The rate of oxygen consumption when sodium chloride was the sole salt in the perfusing fluid was consistently greater than that on a Ringer's solution; this increased oxygen consumption was markedly inhibited by the addition of potassium cyanide 1:10,000 to the perfusing medium. This is in agreement with Warburg's observation. Unlike Warburg, however, we did not find calcium chloride to inhibit this greater oxidation; rather we noted a slight further increase in the oxygen consump-

TABLE 1

Showing the oxygen consumption and change in rate of the isolated terrapin heart when perfused with various fluids

PERFUSING MEDIUM	NUMBER OF EXPERIMENT	O_2 PER GRAM TISSUE	CHANGE IN RATE
		cc.	
Ringer's solution	8	1.38	20–17
Sodium chloride	12	1.89	18–17
Sodium and potassium chloride	6	1.14	23–12
Sodium and calcium chloride	3	2.05	17–17
Dextrose	3	0.93	18–12
Sodium chloride and potassium cyanide 1:10000	3	0.38	

tion. This increase may have been due to the stronger beats exhibited by hearts on sodium and calcium chlorides than by those on a Ringer's solution. We question this explanation, however, since both rate and amplitude of the beats exhibited by hearts on an isotonic solution of dextrose were greatly increased for a considerable time, after which they grew progressively weaker, yet oxygen consumption was greatly depressed throughout the time the hearts were perfused with dextrose alone.

The behavior of the hearts on sodium and potassium chlorides was apparently that of potassium depression. Whether the increased consumption of oxygen when the heart is perfused with sodium chloride alone can be construed as supporting Loeb's theory of a poisoning action upon cardiac muscle appears to us improbable. It is to be noted that those hearts perfused with sodium and potassium chlorides used far less oxygen than did those hearts perfused with a Ringer's solution; yet Baetjer and McDonald (1932) showed that the terrapin sinus brought to complete rest,

electrically and mechanically, by perfusion with sodium chloride alone recovered as quickly and as completely when perfused with a Ringer's solution as did those brought to rest through perfusion with sodium and potassium chlorides; nine sinuses exhausted on one of these two solutions were recovered; no failures to recover were encountered with these solutions. On the other hand, six out of seventeen sinuses exhausted by perfusion with an isotonic dextrose solution failed to recover; oxygen consumption on this solution is even lower than that on sodium and potassium chlorides.

SUMMARY

Thirty-five experiments are reported in which the rate of oxygen consumption of the isolated terrapin heart perfused with a Ringer's solution is:

1. Increased when perfused with an isotonic solution containing either sodium chloride alone or sodium chloride plus calcium chloride. The increased rate upon sodium chloride is shown to be inhibited by the addition of potasium cyanide 1:10,000.

2. Decreased when perfused with an isotonic solution containing sodium chloride plus potassium chloride or with an isotonic solution of dextrose.

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INSENSIBLE PERSPIRATION AND THE GALVANIC SKIN REFLEX

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Psychologists and physiologists recognize the importance of a reliable index of energy transformation in the intact organism. New metabolic techniques are continually being developed, but in many instances very little is known concerning their limitations and what they actually indicate. This is particularly true of the two measures relating to metabolic activity, insensible weight loss and the galvanic skin reflex. That a close relation exists between weight loss attributable to the evaporation of water from the skin or lungs and heat production has been shown by Benedict and Root (1) and by Wiley and Newburgh (2). That the galvanic skin reflex is intimately connected with the water elimination of the sweat glands is shown by the work of Leva (3), Waller (4), Richter (5), Darrow (6) and others, and that it is also closely related to metabolic activity may be inferred from a study by Purdy, Johnson and Sheard (7) and from observations by Davis (8). Notwithstanding this evidence, the conclusion that because these two measures are largely determined by sweat gland activity, they are therefore necessarily high correlated with each other and are equally valid measures of "general metabolism" is open to question.

A reason for not expecting too close a correspondence between these measures is the fact that one bears a less specific relation than the other to local bodily activities. On the one hand, it is questionable whether the local activity indicated by galvanic changes in any single skin area can be relied on as an index of the general metabolism, especially since the activity in one part may vary independently of activity in the rest of the body. On the other hand it is apparent that if the proportion of water evaporated from the various skin areas and the lungs varies with local bodily conditions and with the type of activity engaged in, the total insensible weight loss gives only an indefinite and a highly fortuituous composite of the activities of the different regions of the body.

The present research attempts to throw light on the problem of metabolic measurement and galvanic reactivity through a study of the interrelations between total insensible weight loss as an index of general metabolism and the galvanic skin reflex as an index of the local secretion of perspiration

attributable either to local or to more general bodily activity. Results from tests of from one to two hours' duration on a group of thirteen male subjects under a variety of non-basal conditions will be reported.

APPARATUS. *Galvanic skin reflex.* A form of the Behavior Research Resistance Box (9) was used. This is a Wheatstone bridge having fixed resistance in three arms, balancing 300,000 ohms in the subject's arm of the circuit. Additions or subtractions of 1,000 ohm steps from balance give a scale of galvanometer deflections which may be used in measuring the subject's changes.

Since in this study it was desired to have readings on more than one part of the body, an indifferent electrode (plus) was attached to the ankle with the skin pricked beneath to eliminate the resistance changes in that area and active electrodes (minus)[1] were attached to other skin surfaces. A switch permitted throwing first one active electrode plus a series resistance, then another, into the circuit until the several skin areas had been surveyed. The resistance values of the skin under the respective active electrodes were read in rapid succession from a Leeds and Northrup Type D galvanometer empirically calibrated in 1,000 ohm steps.

Insensible weight loss. Benedict's adaptation of the Sauter balance was used. This is, essentially, a delicate pair of scales, sensitive to 1 part in 1,000,000 on one arm of which is suspended the subject's cot. The scale is accurately balanced at the beginning of the experiment and the subject's insensible loss thereafter is compensated for by the addition of weights on his side of the scale. In determining a possible relation to the galvanic skin response, it seemed necessary that readings of weight loss should be made more frequently than at the 15-minute intervals recommended by Benedict. A means of automatically compensating for the subject's weight loss while maintaining the scale in approximate balance was therefore devised. The compensation device consisted of an electro-chemical system for adding small amounts of water to maintain an approximate balance of the scale. A calibrated burette tube was attached to the subject's side of the scale in such a position that its mouth was opposite the knife edge supporting the subject's cot. A pipette was suspended directly over the mouth of the burette and connected by a tube to a sealed bottle containing water with sufficient addition of sulphuric acid to render it conductive. Electrodes sealed in the bottle generated hydrogen and oxygen when a current of electricity was passed between them. In order to make the amount of water forced out of the pipette and into the burette proportional to the weight loss, four delicate brass springs were arranged, one in front of the other, so that when the scale was thrown slightly off balance by the subject's weight loss the first spring would be pushed against the second by the pointer of the scale, making an electrical contact. If the pressure were further increased, the second spring was then forced against the third, short-circuiting a resistance, and if it were still further increased, the third was forced against the fourth, short-circuiting a second resistance. Once the balance was brought to equilibrium, compensation was automatically made for the subject's insensible weight loss, with only a minimum swinging of the scale. The greater the displacement of the pointer, due to the subject's weight loss, the greater the cur-

[1] These electrodes were relatively non-polarizable (zinc, concentrated zinc sulphate). Evidence obtained since the completion of this study suggests that a gradual drop in resistance due to local effects on the skin under the electrodes would have been less, and higher correlations with insensible loss might have been obtained had we used the plus electrode as the "active" one and employed dilute zinc sulphate of physiological concentration next the skin.

rent, the more gas generated and the more water dropped in the burette. The differences between successive minute readings of cubic centimeters of water in the burette correspond roughly to the subject's weight loss in grams. Save for the exceptional instances when the mechanism obviously failed, this device furnished a fairly accurate index of weight loss during short periods of time.

PROCEDURE. In setting up the experiment we first worked from the hypothesis that a maximum interrelationship between the two measures of reactivity would appear if the subject was studied under widely different stimulating conditions which would carry him from near basal up to maximal activity, and back again to near basal condition. After the subject had rested on the cot from fifteen minutes to half hour, he spent succesive five-minute periods in the following activities: looking at a waterfall illusion, adding, tapping, moderate pulling of a dynamometer, heavy pulling of a dynamometer, responding to word associations, answering intimate questions, receiving electric shocks, receiving electric shocks while pulling a dynamometer, tapping, adding, watching a waterfall illusion, and resting with eyes closed. The humidity was kept relatively low (about 40 *per cent*) and the temperature at approximately 80°F.

This general procedure was employed with four subjects and failed to show any great correspondence between the two measures. Examination of the records suggested that different physiological mechanisms might be responsible for the weight loss during various activities, as for example, evaporation from the lungs in certain activities, and sweating and evaporation on the more active parts of the body in others. Perspiration in the palms of the hands also occurred under special conditions as will be indicated later. Furthermore, the fact that three of the subjects were clothed undoubtedly altered the amount and temporal course of the evaporation of perspiration.[2]

We therefore varied the conditions by imposing various degrees of only one type of stimulation upon the near basal condition, and by using nude subjects. Mild to moderately strong electric shocks were applied to the left leg in the case of nine unclothed men. The total period of observation was approximately the same as in the first experiments, the shocks being gradually increased in intensity and in frequency, and then reduced. In a tenth subject mental arithmetic (addition) of varying difficulty was used as stimulation. In all experiments, minute by minute readings were taken of the total insensible weight loss and of the resistances on the palm, back of hand, wrist or leg.

[2] H. Heller (10) points out that the inclusion of hygroscopic materials in the weighings causes noticeable variations in the amount of recorded insensible loss under basal conditions. F. H. Wiley and L. H. Newburg (2) have shown that there is much closer correspondence between insensible weight loss and changes in environmental temperature and humidity in the nude subject than in one fully clothed.

RESULTS. In table 1 we present the correlation coefficients between total weight loss and average conductance[3] for the various subjects. It will be noted that the values show a wide variation in magnitude and that no single skin area gives consistently higher correlations than any other, although the palms most often gave the highest value. The coefficients are generally higher for nude subjects carried through various degrees of one activitity than for clothed subjects carried through a variety of performances.

In figures 1 and 2 are shown graphs of weight loss and conductance for the various skin areas recorded for two subjects, one of whom gave the

TABLE 1

Rank order correlations between total weight-loss and conductance in various skin areas at two-minute intervals

SUBJECT	CLOTHING	STIMULATING CONDITIONS	CORRELATION COEFFICIENTS				
			Palm R Hand	Back R Hand	R Arm or Wrist	R Leg	L Leg
Co	Clothed	Varied	*0.39*		*0.03*		
Eb	Clothed	Varied	*0.65*	0.27			
Wo	Clothed	Shock	*0.86*	0.85			
Br	Clothed	Adding	0.73	0.71	*0.74*		
Hi	Nude	Varied	*0.78*	*0.78*			
Bo	Nude	Shock	*0.50*			0.46	0.46
Fr	Nude	Shock	0.39	0.21	*0.51*		
Fm	Nude	Shock	*0.50*			0.06	−0.14
Da	Nude	Shock	*0.70*	0.50			
Ke	Nude	Shock	0.74		*0.81*	0.72	
Cr	Nude	Shock	0.65		0.72	*0.83*	
Vi	Nude	Shock	*0.91*		−0.40		0.55
Bn	Nude	Shock	*0.97*	0.94	0.95		

highest correlations and the other of whom gave about average results. The graphs are plotted for the total weight loss, and the average conductance of successive two minute periods, readings for odd and even minutes being combined to give greater smoothness to the curves. Inspection of the curves indicates considerable independence of the reactions shown by the various measures. Study of the graphs and the scatter diagrams of the various subjects gave clues as to the nature of the relationships between the various measures, which will be treated topically.

[3] Evidence that the amount of local perspiration tends to vary as the reciprocal of the resistance measurements (conductance) makes units of conductance desirable for the present comparison. Resistances have, therefore, all been translated into microhmos (the reciprocal of the megohmic resistance).

On last line of table 1, page 58 (February, 1935), Volume 111, read 91, 74, 75, instead of 97, 94, 95.

The significance of reactions in palmar and non-palmar skin areas. Inspection of figure 2 gives the impression that the higher rank correlation with weight loss shown by the palm did not in this instance necessarily indicate the greater degree of general parallelism with weight loss. The same impression was conveyed by the graphs of several other subjects. This is important in view of the evidence from this same material (12)

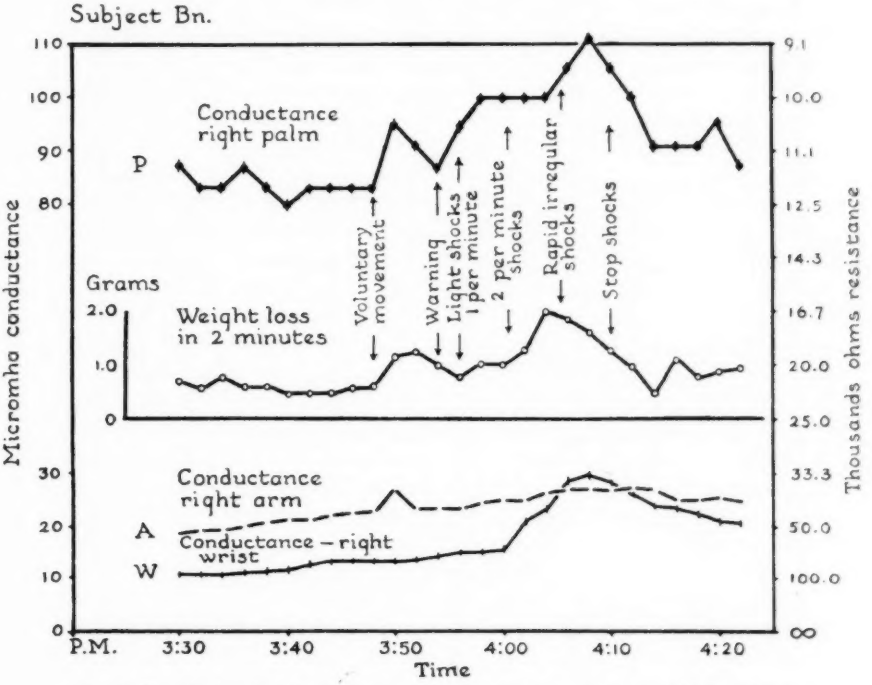

Fig. 1. Graph showing the relation of conductance to grams weight loss for successive two minute periods. Total weight loss and average conductance of odd plus even minutes are here plotted in the interest of reliability and smoothness of curve. This subject gave the highest rank correlations between the records of conductance and total insensible weight loss.

that the larger reactions in the palms do not occur in situations involving the greatest output of energy, but in situations involving increased "alertness" while those in the non-palmar regions are more often related to "muscular activity" and energy production. Reactions occurring after warning and during "anticipation" or "apprehension" of the impending stimuli, and on awaking from sleep show palmar predominance of the reaction to marked degree. Reactions occasioned by muscular activity on the con-

trary, generally show a predominance of the non-palmar responses. This supports the hypothesis that while non-palmar sweating is primarily thermoregulatory in function, that on the palms and the soles of the feet is for the purpose of maintaining the pliability and adhesiveness essential to tactual acuity and good grip upon objects. Sweating in these latter areas in situations involving increased "alertness" may be considered as a

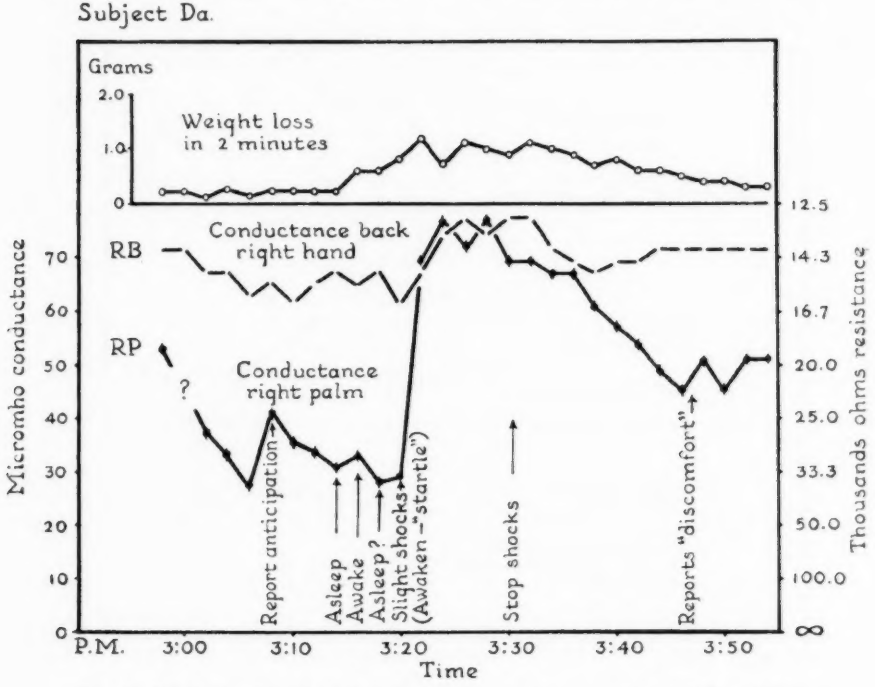

Fig. 2. Graph showing the relation of conductance to grams weight loss for successive two minute periods, for subject who gave correlations of about average magnitude. Note that although the palm gave the higher rank correlation, the back shows the closer general parallelism with total insensible weight loss.

part of the general mechanism by which the organism is mobilized for emergency adjustive response.

Latency of galvanic and weight loss changes. Under conditions causing an increase in reactivity, increments in skin conductance tended to precede increments in weight loss. The tendency was greatly enhanced when the subject was fully clothed. This suggests that the conductance increment occurs simultaneously with the condition causing increased sweating, while the weight loss increment follows only when the sweat is evaporated. On

the few occasions when weight loss preceded the galvanic change, it seems likely that there was an immediate local secretory effect of the activity and a delayed effect in the more distant parts of the body to which the electrodes were applied. Under conditions favoring a decrease in reactivity, decrements in skin conductance also tended to precede decrements in the rate of insensible loss; although with the subject nude, the reverse order sometimes obtained, the rate of insensible loss returning to the resting level earlier than the corresponding decrease in skin conductance appeared.

Effects of different types of activity on galvanic and weight loss changes. When the subjects were carried through a widely varying series of activities certain striking correspondences and differences in the course of the several physiological measures appeared. Rest while looking passively at a moving waterfall illusion was regularly accompanied by a decrease in both conductance and weight loss. Mental arithmetic (addition) after a relatively quiescent state, caused a large increase in palm conductance as well as an increase in weight loss. Galvanic effects in the back of the hand were definitely small for this activity. When the mental arithmetic followed a period of more strenuous activity the galvanic effects were more variable. Tapping with the left index finger and gripping a dynamometer had varying effects, apparently depending on the intensity of the activity itself and on the degree of activity immediately preceding. Disturbing word associations and sex questions were attended by irregular changes in the rate of weight losses and in the conductance of the palm. Instances in which subjects were judged by their breathing and closed eyes to be asleep were uniformly characterized on waking by a marked increase in palm conductance, and often by but slight increases in rate of weight loss. In several instances apprehension regarding the character of the next stimulus caused reactions, especially in the palm, which were not temporally associated with any change in the prevailing stimulating condition. For example, subject *Br*, who was afraid he was to be given an electric shock, showed a large drop in palm resistance attended by moderate increase in weight loss during a period of minimal external stimulation. The nude subject, *Hi*, showed but a slight increase in the correspondence of the galvanic and weight loss measurements over that shown by the clothed subjects.

Galvanic and weight-loss changes during variations in the degree of a single activity. When a qualitatively uniform stimulus situation was presented to nude subjects after a period in a near-basal condition of rest, marked correspondences appeared between changes in conductance of the skin and total weight loss. Progressive increments in the intensity and frequency of electric shocks followed by progressive decrements or by cessation of the stimulus, caused the rate of weight loss to increase and then to decrease. This was also true when arithmetic problems were substituted for shocks.

The temporal course of weight loss changes was paralleled to some degree by the changes in conductance level in all subjects. However, in only one instance did a single conductance measure adequately duplicate the course of weight loss changes. In seven other subjects, resistance changes in some one skin area tended to parallel weight loss changes more closely than did conductance changes in other areas. That no particular skin area was more favored is shown by the fact that in subjects *Br* and *Ke* the right arm gave slightly the better correspondences while the right palm was definitely superior in *Vi*, *Eb*, and *Fm*, and the right leg for *Cr*. In the case of *Bo* no obvious parallelism was found between the conductance of any of the three skin areas explored and the total insensible weight loss, although the correlation coefficients between the measures are of about average magnitude. In several instances almost perfect correlations between the two measures could be worked out by utilizing different parts of different conductance curves. Apparently no single skin area can be counted on to give exact correspondence to the total energy turnover as revealed by weight loss.

Effect of air circulation and respiratory exchange upon the galvanic-weight loss relationship. The effect of decreasing the environmental temperature and increasing the air circulation was studied in two clothed subjects. In both cases there was an initial drop in palm resistance (due to introduction of a new auditory and cutaneous stimulus?) followed by a rising resistance in back and palm (due to decreased sweating?). Weight loss records showed a temporary increase (due to increased circulation of air?) followed by a large decrease (due to decreased sweating?). In another subject a comparison was made between the degree of conductance-weight loss correspondence under (*a*) conditions favoring maximum evaporation of water from the skin and minimum evaporation from the respiratory passage, and (*b*) conditions favoring minimum evaporation from the skin and maximum evaporation from the lungs. As would be expected, the correspondence of the two measures was much better under condition *a*. With all skin areas including the hands and face covered and with the subject breathing rapidly and deeply, resistance changes showed no apparent relation to weight loss changes. The data on these special conditions have been omitted in determining the correlation coefficients of table 1.

SUMMARY AND CONCLUSIONS

Data obtained upon thirteen subjects show occasional striking correspondences as well as many discrepancies between measurements of skin resistance (translated into *conductance*, the electrical unit which corresponds to changes in the amount of locally secreted perspiration) and the rate of total insensible weight loss. In different individuals skin conductances at corresponding points do not always show the same relation to the rate of

total weight loss. In the same individual under varying conditions, first one skin area and then another may show the greater parallelism with weight loss, as if one part of the skin and then another were playing a primary rôle in heat elimination. It is evident that measurements of skin resistance (conductance) may not, at least by the methods here employed, be used as an index of the general insensible weight loss, and, indirectly, of the metabolic level of active persons. Conversely, since the human organism presents different levels of metabolic activity in its several parts, and since increase in activity in one part may be counteracted by decrease in another, it is obvious that the occurrence of a metabolic increment or decrement under a given condition is highly fortuitous, and its value for activity studies always subject to question. Whether or not these inferences are applicable to the problem of metabolic measurement in persons in a "basal" condition is not answered in this investigation.

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EFFECTS OF CARBON DIOXIDE UPON URINE FORMATION AND GLOMERULAR BLOOD FLOW

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While studying urine formation in frogs a peculiar effect of lack of oxygen was found (Adolph, 1934a). Whenever frogs were subjected to a low tension of oxygen or whenever breathing ceased because of injury, no urine was formed. Upon examining the exposed kidneys of pithed frogs a sufficient reason for this was discovered, namely, that in the absence of oxygen blood ceased to flow through the renal arteries and glomeruli. When a small tension of oxygen was present some glomerular activity persisted, but all rates of blood flow below the maximal could be identified under suitable circumstances.

A search was made for other means of modifying the activities of the kidneys over short periods of time. It was found that carbon dioxide might inhibit urine formation even when plenty of oxygen was given. Studies have been made, therefore, both on intact frogs and on pithed frogs which had their kidneys exposed, in the effort to analyze this phenomenon. In many experiments parallel observations have been made upon the urine production of frogs with one ureter cannulated at the same time that the rate of blood flow in the glomeruli was observed. In this way certain quantitative relationships have been established.

The observations were not concerned with the changes of concentration that solutes undergo as they pass from the blood stream to the urine, but with the regulation of the excretion of water. The particular problem which the present experiments serve to elucidate is: to what extent do circulatory changes control the rate of urine formation?

Intact frogs. There are two ways of comparing the urine production under different experimental conditions. The amount of urine collected may be calculated relative to the intake of water during the same period of time; or it may be calculated relative to the amount collected from the same or other control frogs that were in room air or in 100 per cent oxygen. The former is the more accurate in the case of intact frogs; the latter has to be employed in operated frogs.

The species *Rana pipiens* was used in all experiments. The rate of urine

formation was first studied in intact individuals. Frogs were weighed, their cloacas were ligated so that the urine formed was retained over a known period of time, at the end of which the urine was liberated and the loss of weight ascertained.

Gas mixtures were made up in cylinders under pressure, and samples of each were analyzed. Frogs were exposed to the mixtures in sealed pint

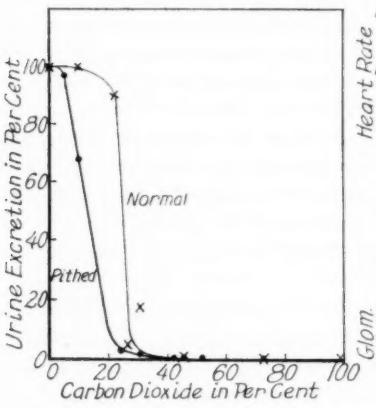

Fig. 1

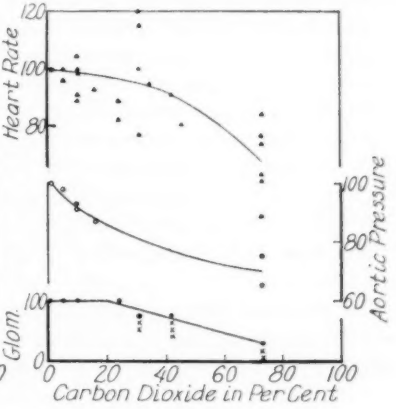

Fig. 2

Fig. 1. Mean relative rates of urine formation at diverse tensions of carbon dioxide. Oxygen always constituted the remainder of the gas mixture at atmospheric pressure to which the frogs were exposed, at 20° to 24°C. × normal frogs (average of 3 to 8 measurements); ● pithed and opened frogs with one ureter cannulated (average of 2 to 5 measurements). In each case the rate of urine formation in control individuals or in control periods when exposed to room air was taken as 100 per cent, except where pithed frogs were exposed to 5, 10 and 24 per cent carbon dioxide, when pure oxygen was taken as 100 per cent.

Fig. 2. Circulatory changes in pithed frogs at diverse tensions of carbon dioxide (20° to 24°C.). The final value prevailing at the termination of each exposure to carbon dioxide, which usually lasted 15 to 30 minutes, is given as per cent of the value just previously prevailing in room air. The glomerular activities are the averages of numerous tests; dots indicating the percentage of observed glomeruli in which blood flowed, and the crosses indicating that the flow was slower than in pure oxygen.

jars, containing 50 to 70 cc. of tap water, and provided with inlet and outlet tubes. From 1500 cc. to 5000 cc. of the gas mixture were passed into each jar during 10 to 20 minutes, after which the jar was closed off under atmospheric pressure for about two hours.

In tensions of carbon dioxide above 30 per cent of one atmosphere no urine was excreted. At 30 per cent, urine was formed to a small extent in some individuals. At lower percentages considerable urine was produced

and at 10 per cent the rate of formation was equal to that in room air or in pure oxygen. The mean rates of urine excretion are indicated in figure 1.

Various factors were tested to see whether they modified the inhibition of urine formation by carbon dioxide. In four frogs both kidneys were denervated, so far as this is possible by cutting strands of the sympathetic system. The response to 30 per cent carbon dioxide was unchanged. In six frogs both renal portal veins were ligated; again anuria was produced by 30 per cent carbon dioxide. Fluid was administered subcutaneously by injecting 1 per cent urea solution in three frogs; again no urine was excreted in the presence of 30 per cent carbon dioxide.

Another acid was tested to see whether the effect of carbon dioxide could be duplicated. Acetic acid was added up to a concentration of 0.02 M to the water in which the frogs were immersed. Whenever the frogs survived this medium, urine was formed to nearly the normal extent (mean of 9 frogs, 91 per cent of the normal rate), in spite of the fact that there was an additional gain of net body weight as long as the acid medium was present.

The character of the urine that was formed in the presence of low tensions of carbon dioxide and in the recovery period subsequent to the inhibition of urine formation was studied in two respects. First, it was found that chloride was present in the usual small concentrations characteristic of frogs not subjected to carbon dioxide. Second, it was found that protein was not present in the urine to any greater extent than in frogs not subjected to carbon dioxide. Hence so far as the present observations go, there were no marked effects upon the activities of the kidneys themselves outside of the fully reversible inhibition of water excretion.

Recovery after subjection to carbon dioxide was studied by ligating the cloacas a second time as soon as the frogs were returned from an atmosphere of carbon dioxide to an atmosphere of room air. In every case urine was formed at more than the normal rate within two hours after exposure to carbon dioxide. Within four hours the net weight of the body had always returned to normal, and the amount of urine obtained in the recovery period was sufficient to make up for the carbon dioxide period during which no urine had formed. Also, in frogs that were not ligated, after subjection to carbon dioxide it was apparent that recovery of renal function was almost immediate, since they started losing weight within a half hour after return to room air.

In general the time required before urine once more began to be formed and before net body weight began to be lost was a function of: first, the tension of carbon dioxide to which they had been exposed, and second, the duration of the exposure. The breathing often ceased in the presence of carbon dioxide of 30 per cent or higher concentration. The cessation of breathing in itself could inhibit urine formation at the temperatures at

which these experiments were conducted (Adolph, 1934b). However, numerous instances were observed in which breathing persisted and yet urine formation ceased. In this way it became evident that the effect of carbon dioxide was not due to this factor alone, and additional reasons for so believing were obtained upon non-breathing pithed frogs, as will be noted below.

Glomerular activity. The circulation in the exposed kidneys was observed in frogs with the entire brain pithed and placed in a gas-tight chamber as previously described in the experiments on lack of oxygen (Adolph, 1934a). Considerable areas of the skin were covered by cotton soaked in water. As soon as carbon dioxide displaced the air of the chamber, glomerular blood flow in the exposed kidney was sometimes stopped. However, this stoppage was not a constant factor. Often stoppage was observed in 30 per cent carbon dioxide, while in other frogs it was not observed in 70 per cent carbon dioxide. On the average it was observed oftener, however, in the higher tensions. A single frog appeared to respond in a more consistent manner. Again, some particular glomeruli regularly stopped in response to carbon dioxide while others did not. Evidently there were inherent differences in the blood flow to different renal units as well as differences in animals. The average results obtained on the glomeruli observed are indicated in figure 2.

Certain observations were made to find why the blood flow through the glomeruli either ceased or became slow in the presence of carbon dioxide. Lack of oxygen had been found to constrict renal arterioles; and occasionally a similar constriction was observed with carbon dioxide. This, however, was rare and it appeared that the diminution in blood flow was ordinarily passive so far as the *visible* arterioles of the kidneys were concerned. Indirect evidence will be added in a later section which indicates that carbon dioxide causes a constriction of blood vessels at some point between the aortas and the glomeruli.

To find what rôle the systemic circulation played, observations were made upon the general changes in the vascular system occurring in the presence of carbon dioxide. These observations consisted of measurements of arterial blood pressures and of cardiac pulse rates during subjection to carbon dioxide (fig. 2). In figure 3 a representative series of tests is shown in which a pithed frog with one aorta cannulated was subjected to various tensions of carbon dioxide. It will be seen that the arterial pressure invariably fell in high tensions of carbon dioxide, and even low tensions affected it slightly. The heart rates were proportional to the arterial pressures, which probably indicates that the falling pressure was primarily due to embarrassment of the heart. The embarrassment may possibly have been due to factors other than a change of acidity in the heart muscle. Because of the correlation between heart rates and aortic pressure, average

changes in arterial pressure could be assumed from the changes in heart rate which were observed in a much greater number of experiments.

In the formation of urine, the quantity which counts most, according to prevalent conceptions, is not the arterial pressure but the pressure of the blood within the capillaries of the glomeruli. This pressure has been measured in pithed frogs by Hayman (1927), and on the average amounts to between 54 and 85 per cent of the systolic pressure in the aorta. In the present experiments 30 per cent carbon dioxide, which stopped urine formation and often stopped glomerular blood flow, diminished the arterial pressure 10 to 20 per cent below the normal. A diminution of 20 per cent in the glomerular capillary blood pressure of itself could hardly account for stoppage of urine formation since the absolute pressures probably varied enormously in diverse individuals that were equally affected by 30 per cent

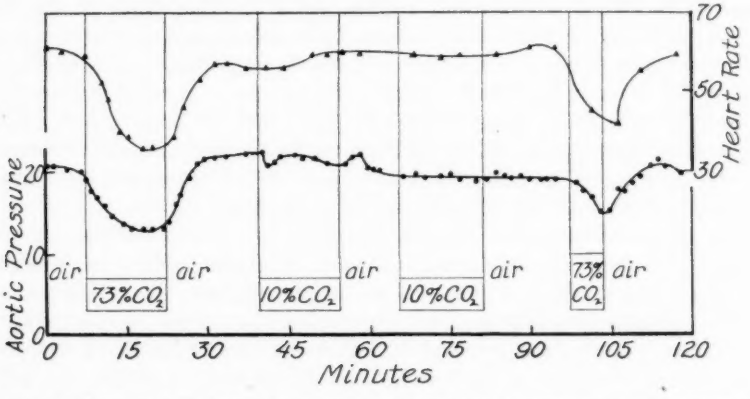

Fig. 3. Heart rates in beats per minute and mean aortic pressures in millimeters of mercury, observed in pithed frog *Qj* at 21°C.

carbon dioxide. In fact, assuming that the capillary blood pressure is the chief factor forcing water from the blood within the Malpighian capsules of the kidney, a 20 per cent decrease in the pressure in the glomeruli would in most instances still leave the pressure well above the opposing colloid osmotic pressure of the blood plasma. An alternative seems to be, therefore, that the pressure in the glomerular capillaries is much reduced relative to the pressure in the aorta.

The observation must be emphasized that the flow of blood through the glomerular capillaries often persisted under conditions in which urine formation stopped. Hence the *rate* of blood flow is not the chief factor in urine formation, and it becomes likely that the local *pressure* is of much more importance.

Urine formation in pithed frogs. In order to correlate accurately the

changes seen in glomerular blood flow with simultaneous rates of urine formation it was necessary to study both these quantities in the operated animal. In male frogs one ureter was cannulated at the same time that the exposed kidney from which the ureter led was kept under observation. To measure urine formation over very short periods of time a cannula of uniform bore was used and successive positions of the meniscus were read off along its length. Thus it was possible to measure the rates of urine excretion over intervals of a minute, or less, and to find out what progressive changes occurred in the rates. Whenever the cannula filled, it was

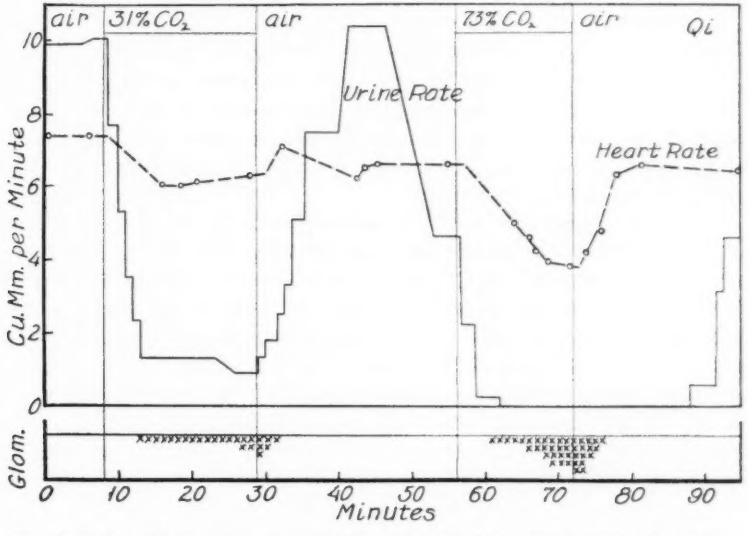

Fig. 4. Rates of urine formation observed in pithed frog *Qi* at 23°C. Heart rates are represented in beats per one-tenth minute. Glomerular activities are represented by plotting the number of those glomeruli that were being observed (six) which showed blood flowing through them. The crosses below the line indicate out of this number those that conducted blood only slowly.

emptied by inserting a narrow pipette through a temporary opening in the side of the gas chamber.

A representative experiment is shown in figure 4. A single frog was successively exposed to various tensions of carbon dioxide, recovery occurring between exposures when room air passed through the chamber. As soon as carbon dioxide was introduced into the chamber containing the frog, urine formation either slowed or ceased entirely, depending upon the tension of carbon dioxide. The heart rate was always reduced, and presumably therefore the general circulation was somewhat impeded, but the

protocols clearly indicate that urine excretion ceased more promptly than the heart rate and aortic pressure fell off. The glomerular circulation slowed somewhat in each exposure, but in the particular experiment shown in figure 4 blood continued to flow through all the glomeruli that were under observation, though at reduced rates, even during exposure to 73 per cent carbon dioxide.

The mean changes in rates of urine formation on the part of pithed frogs are shown in figure 1. It is evident that a smaller tension of carbon dioxide was required to inhibit urine formation, in the non-breathing pithed frog that had a ureter cannulated and the viscera exposed, than in the normal intact frog. This indicates that the limiting factors of urine formation were not alike in the operated frog and in the normal one in the region of 10 to 25 per cent carbon dioxide tensions. The difference may have been due to a number of factors, notably the oxygen content of the blood. In other tensions the effects were similar, so that the belief might be justified that most of the circulatory changes observed in the pithed frog occurred equally in the normal intact frog.

With the arrangements used for exposing the frogs to gas mixtures, the new gas surrounded the frog within one minute. The chamber used had a gas space of about 300 cc. and gas entered at first at the rate of 400 to 600 cc. per minute. Access of carbon dioxide mixtures to the frog was often signalized by muscular movements; part of these were prevented by previously cutting all sciatic nerve trunks. The effect upon urine formation was usually evident within 2 minutes, and where the excretion was completely inhibited cessation occurred within 8 minutes at the most. The latency in the effect was therefore extremely brief; in fact, considering the elasticity of the tissues within the kidney, the response as observed was about as prompt as could be expected if it were instantaneous.

Recovery of urine formation after exposure to carbon dioxide was very prompt in some instances, particularly where the urine formation was not completely inhibited but merely required to be speeded up. In other instances the latent period was surprisingly long, and cases are available, as in figure 4, where 15 to 25 minutes were required before any urine was formed. As soon as urine had again begun to flow, the rate of its formation gained speed very rapidly, and frequently a definite diuresis could be observed (fig. 4) which lasted many minutes but which spontaneously ended. This diuresis in the pithed frog after a short exposure to carbon dioxide is comparable, so far as can be ascertained, with the diuresis which followed the prolonged exposure to carbon dioxide in the intact frog. Both the time required for recovery and the amount of diuresis seemed to be related to the tension of carbon dioxide and the duration of the exposure, as was the case in the intact frog. The mechanism whereby the urine deficit is made up is possibly circulatory, and specifically the same as for reactive hyperemias in general.

In a few instances tests were made to see whether the intravenous introduction of fluid into the frog would overcome the anuria caused by the carbon dioxide. When the anuria was complete it did not appear possible to promote urine formation. When, however, anuria was not complete, even though it might have reduced the urine formation to only 2 per cent of the normal, it was possible to promote urine formation temporarily by an injection of salt solution containing a small dose of adrenaline (fig. 5). The extra urine formation thus induced, however, was of relatively short duration and the rate returned to its original value of 2 per cent or less,

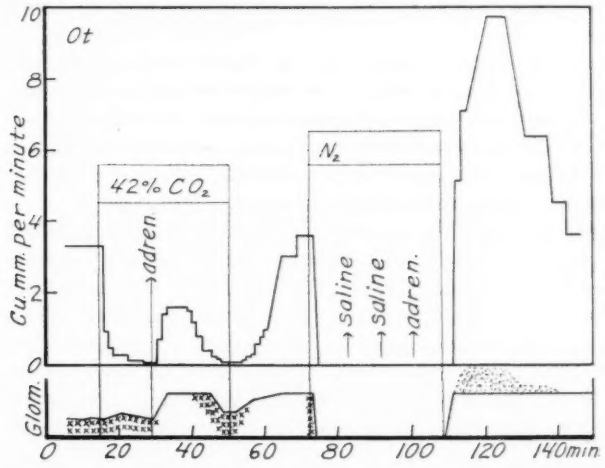

Fig. 5. Rates of urine formation observed at 21°C. in pithed frog *Ot* weighing 30 grams. Urine formation was slowed by exposure to 42 per cent carbon dioxide but was temporarily hastened in its presence by an intravenous infusion of dilute adrenalin solution (0.8 cc. of 1:500,000). When excretion was later stopped by exposure to lack of oxygen, similar infusions (0.8 cc. and then 1.0 cc. of 0.1 M NaCl solution, 0.5 cc. of 1:200,000 adrenalin solution) had no effect. Glomerular activities are represented as in figure 4, as *slow* by crosses below the line and as *excessive* by dots above the line.

until the carbon dioxide atmosphere was replaced by air. The changes in flow of blood through the glomeruli that accompanied the temporary diuresis are also recorded in figure 5. Similar changes in glomerular blood flow coincident with diuresis have been observed by Tamura (1927a).

From the present few observations upon diuresis it appears that urine formation is impossible when the glomeruli have no blood flow at all, but when some blood flow, small though it may be, is maintained, the rate of urine formation may be varied by increasing the blood volume of the body as a whole. This observation again points to a local control of blood flow

within the kidney during oliguria, which shuts off the arterial blood from the glomerular capillaries more or less promptly and permanently.

COMMENT. The experiments furnish a precise method of controlling the rates of urine formation in the frog over brief periods of time without imposing any irreversible changes. Whereas injection of fluid into the blood requires manipulation and involves change of blood volume, exposure to modified atmospheres of carbon dioxide, so far as is known, does nothing to change permanently the state of the frog. In the pithed frog, as many as 25 changes of gaseous atmosphere have been imposed during the course of a day, and at the end of this time the frog was apparently in the initial condition.

The rates of urine formation vary evidently with extreme rapidity in response to conditions imposed. By other methods, conditions must be imposed for an hour or more at a time in order that effects may be measured. By this technique, however, effects can be traced from minute to minute. This is very important when it is desired to study in comparison the successive influences of experimental circumstances.

The anuria caused by exposure of frogs to carbon dioxide has been analyzed by direct study of the kidney, and is ordinarily correlated with the observed phenomena of blood flow; but it was proved that the persistence of blood flow alone does not insure urine formation. Blood flow is the only quantity directly observed by the methods so far employed upon the glomeruli of the kidneys; but it is evident that the retardations of blood flow that were observed were not the causes of anuria; rather they were evidences of other events in the organ.

A simple hypothesis is that these other events that are crucial in the rate of urine formation are changes of glomerular capillary pressure. The possible rôle of the pressure of blood in the glomerular capillaries in bringing about the filtration of urine was pointed out by Ludwig (1844). In the frog this circulatory factor was given new significance by the observations of blood flow made by Richards and Schmidt (1924) and of local pressure made by Hayman (1927). Tamura (1927b) objected that the cessation of glomerular blood flow in response to ureter obstruction in the frog is too prompt to be caused by the obstruction's overcoming the vascular pressures that he supposed were available, but precise support for this conclusion does not exist. In fact, the excretion of water in the frog, so far as it is now understood, can be fully accounted for by the excess of hydrostatic over osmotic forces furnished by the circulatory system.

The lowering of glomerular capillary pressure that would be predicted from the observed aortic blood pressure under the influence of carbon dioxide was found to be insufficient by itself to cause the changes of urine formation that were found. But local pressure changes could be effective; they may be mediated by either of two vascular regulators, because two

sets of arterioles exist in the kidney. Constriction of the afferent arterioles would be expected to lower the pressure in the glomeruli without necessarily any lowering of pressure in the systemic arteries. On the other hand relaxation of the efferent arterioles would lower the glomerular pressure without any necessary change in the afferent arterioles, although dilatation of efferents alone would enable the blood to flow faster and not slower through the glomeruli. It is possible that both these events occur, and in varying proportions. Even constriction of both the afferents and the efferents might produce the results observed. It is obvious that the situation has numerous complications, and several types of vascular responses could produce anuria.

The hypothesis could be offered that carbon dioxide somehow narcotized or otherwise inhibited water-secretory activities in the renal epithelium. No facts are available with respect to renal tissue that lend support to such a concept, but carbon dioxide in tensions similar to that which suppresses urine formation has been found to depress various functions of other tissues, as illustrated by the rate of oxygen consumption in nerve (Root, 1932). Although the analysis of the circulatory changes is incomplete, yet the occurrence of such changes is qualitatively known, while activities of renal epithelium with respect to water excretion, and their modifications, are wholly unknown.

SUMMARY

1. High tensions of carbon dioxide tend to inhibit urine formation in frogs. To produce apparently the same effect, slightly higher tensions are required in the intact frog than in the pithed frog which is not breathing and has the viscera exposed.

2. The glomeruli are seen to conduct blood at slower rates in high tensions of carbon dioxide and often the flow through them is stopped entirely. The reduction of flow is not fully accounted for by the slight decreases of arterial blood pressure and heart rate which were observed.

3. Exact correlations are found with great frequency between the flow of blood in the glomeruli and the simultaneous production of urine in the kidney with its ureter cannulated. The latent periods for the onset of anuria are very brief, and the recovery periods after anuria are usually brief but may extend over 25 minutes.

4. It has been proved that blood may flow through the glomeruli while urine ceases to be formed. Evidently the rate of blood flow is less important in urine formation than some other factor which is perhaps the pressure of the blood in the glomerular capillaries.

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OXYGEN TENSION AND URINE PRODUCTION IN FROGS

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These experiments developed from the observation that asphyxia stopped the formation of urine in frogs. That observation, made upon intact frogs, led to the examination of the blood flow in the glomeruli of operated frogs (Adolph, 1934a), and perfect correlation between stoppage of blood flow and the anuria was found. The question then arose whether the operation required to expose the kidney modified the condition of the animal, and hence it became desirable to observe simultaneously in one and the same animal the rate of urine formation and the flow of blood through the glomeruli. Whereas asphyxia or complete lack of oxygen almost invariably stopped all flow of blood through the glomeruli, it soon became apparent that partial asphyxia had profound effects too, in spite of the fact that the flow of blood was not stopped. A second means of correcting for the effects of operation has also been utilized, namely, that of comparing the rates of urine formation in intact frogs and in pithed but unoperated frogs, as well as in pithed frogs with kidneys exposed, when subjected to the same oxygen tensions.

The operated frog. The brain of a frog was pithed and the frog was placed in a gas-tight chamber. One ureter was cannulated and the rate of urine excretion was measured by reading off the successive positions of the urine meniscus in the cannula (Adolph, 1935). The kidney from which this ureter led was prepared for observation of a number of its glomeruli. The frog was successively subjected to various tensions of oxygen from 0 to 100 per cent of an atmosphere by passing mixtures that had been prepared from compressed oxygen and compressed nitrogen through inlet and outlet tubes sealed into the chamber. The frog was not breathing, so that the access of oxygen to the blood occurred entirely through the skin and other exposed surfaces. Cotton soaked with water covered considerable areas of skin in order that water would be taken into the body at all times.

It will be seen in figure 1 that when a low tension of oxygen was administered the rate of urine formation fell rapidly to zero. The decrease in rate was very prompt, beginning in about 3 minutes and ending in about 10 minutes. When 100 per cent oxygen was again supplied, a latent period

of 3 to 5 minutes was followed by a rapid increase in the rate of urine production. Simultaneous observation of the glomeruli sometimes showed a certain slowing of blood flow through them, followed in the lowest tensions of oxygen by complete cessation of this flow. As was previously described this cessation of flow was due to a complete constriction of the renal arterioles. At other times, however, the rate of blood flow was not correlated with urine production. Thus (fig. 1) in 21 per cent oxygen the flow was actually increased just at the time that production decreased; at 140 minutes the flow decreased temporarily while production increased. Simultaneous measurements of heart rate showed a slowing of the heart in

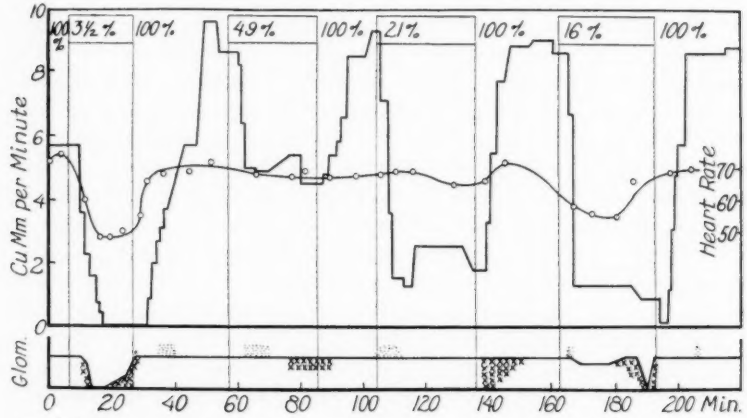

Fig. 1. Rates of urine formation, pulse rates, and glomerular activities in frog *Qm* at 21°C., successively subjected to various tensions of oxygen. Heart rates are in beats per minute. Glomerular activities are expressed as follows: Five glomeruli were watched; the number that had blood flowing through them is indicated by the solid line; flow distinctly slower than normal by crosses below this line; flow faster than normal by dots above this line.

low oxygen tensions which was nearly always accompanied by reduction of arterial blood pressure.

Throughout the experiments 100 per cent oxygen was taken as the basal condition, and to it the frog was usually returned after subjection to another tension of oxygen. It is evident (fig. 1) that room air (21 per cent oxygen) reduced the rate of urine formation considerably, and that even 49 per cent oxygen inhibited urine formation somewhat. The quantitative relationships are illustrated in figure 2. While the rate of urine formation varied in different frogs, and even in the same frog at different times, it is evident that the mean rate of urine formation was less, the smaller the tension of oxygen. The average rates are fitted best by an S-shaped curve, with a sharp decrease between 45 and 15 per cent oxygen. There was

evidence that in a freshly prepared frog there was less difference in the rate of urine formation whether the frog was exposed to air or whether it was exposed to 100 per cent oxygen, and the average relationship illustrated is hardly to be taken as representative of the quantitative comparison in any particular instance. The S-shaped curve in figure 2 appears to be comparable to the curve of Bastert (1929) for the rate of oxygen absorption from the closed-off lungs of pithed frogs. Both curves resemble the

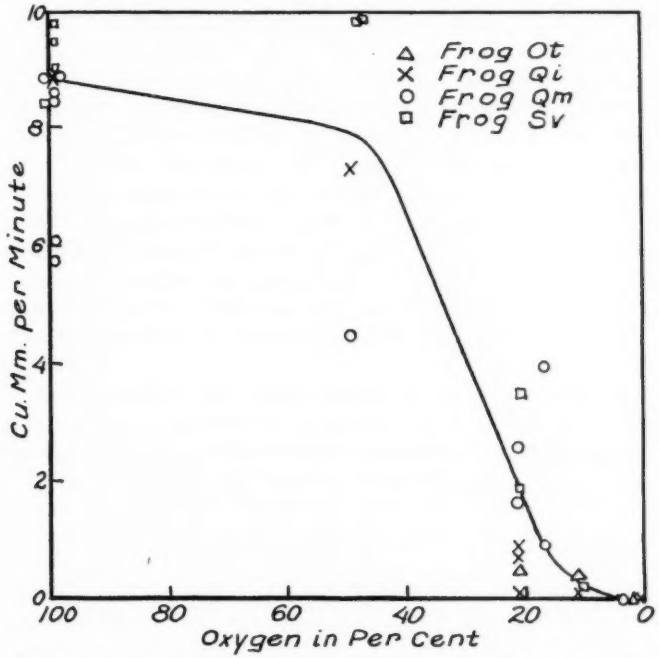

Fig. 2. Rates of urine formation in four pithed frogs at 21° C. to 23° C., exposed to various oxygen tensions. One ureter was cannulated, and the rates plotted were those shown at the end of 20 to 30 minutes of exposure.

oxygen dissociation curve for blood, and might mean that the rate of urine production (and of oxygen consumption) in the non-breathing pithed frog is proportional to the oxygen content of the blood rather than to the oxygen tension.

The rate of urine formation in 100 per cent oxygen was about as rapid as that found in a normal intact frog. This was perhaps a coincidence rather than an indication of maximum function on the part of the kidney, since pithing increased the rate of water intake over that in the normal

intact frog and thereby sometimes increased the rate of urine formation above that of the normal intact frog (Adolph, 1934b).

Following exposure to a low tension of oxygen in which formation of urine was markedly or completely inhibited, diuresis often occurred. This was comparable to the diuresis obtained in the intact frog after a prolonged exposure to lack of oxygen (Adolph, 1934a). It was most marked after complete inhibition of urine formation and was very small after partial suppression of urine formation.

The diuresis was often accompanied by a very rapid flow of blood through the glomerular capillaries, this flow being considerably faster than the normal flow in the same medium (100 per cent oxygen). In the lower tensions of oxygen the glomerular flow was usually observed to be slower than normal. It is, however, very difficult to compare the rates of flow by mere qualitative observations. The blood flow could be accurately measured only in one respect by this kind of observation, which was to count the number of glomeruli in which blood flow ceased. The average numbers that were found active in the various tensions of oxygen are indicated in figure 3. It is evident that it was very common to find the flow stopped in glomeruli when the frog was subjected to 0 to 2 per cent oxygen, but very uncommon to find glomeruli inactive in tensions of 4 per cent or higher.

As was noted previously (Adolph, 1934a), the limiting tension of oxygen that allowed urine to be formed in the normal frog was the same as the limiting tension that allowed blood to flow through the glomeruli in the pithed frog. It now becomes evident that this similarity of limiting gas tensions was quite accidental, for blood often flowed, yet urine was not formed, in the pithed frog at tensions of oxygen higher than 2 per cent of an atmosphere.

Changes in the systemic circulation were measured in various tensions of oxygen. By cannulation of one aorta, arterial blood pressures were observed, and it was ascertained that the changes were negligible in the pithed frog subjected to tensions of oxygen between 100 per cent and room air. In 11 per cent and 0 per cent, however, the arterial blood pressure fell considerably, as is indicated in figure 3. Heart rates were observed in a great many experiments, and the changes in heart rate, as shown in figure 3, were roughly parallel to the changes in arterial pressure. Possibly some lack of parallelism occurred in tensions of oxygen between 2 and 16 per cent; however, in general, the mean pressure in the artery might be inferred from the change in heart rate as tensions of oxygen were varied. But, as was previously reported, the modifications of the systemic circulation did not account for all of the changes in the flow of blood through the glomeruli. These changes of flow were much more severe than any that could be seen in other parts of the body. For instance, the flow of blood

in the renal portal systems was not visibly diminished in low tensions of oxygen, except possibly to a small degree in the complete absence of oxygen.

Normal frog. Having found that the pithed frog was greatly at the mercy of oxygen tension in respect to urine formation, measurements were made on the normal frog and on the frog that was pithed but not operated upon. Both were studied by ligating the cloacas for known periods of time and measuring the rates of urine accumulation. The average effects

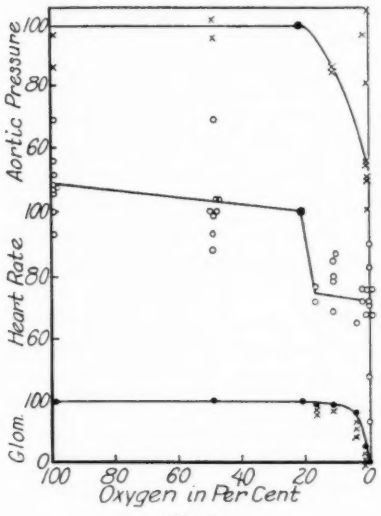

Fig. 3

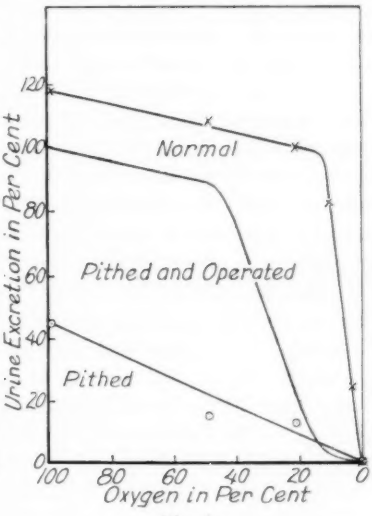

Fig. 4

Fig. 3. Correlations of oxygen tensions with mean aortic pressures, heart rates, and glomerular activities in pithed frogs at 21°C. to 24°C. Each value is expressed in per cent of the value just previously observed while the same frog was surrounded by room air.

Fig. 4. Mean rates of urine production at 21°C. to 23°C. at various tensions of oxygen. For the normal and the pithed frogs, the rate shown by the normal frogs in room air is taken as 100 per cent. For the pithed-and-operated frogs with ureter cannulated the curve is copied from figure 2; and the rate shown by the same frogs in pure oxygen is taken as 100 per cent. In absolute amount this rate for one kidney happens to be about equal to that for both kidneys of the normal frog in air.

of the various oxygen tensions upon the normal frog are indicated in figure 4. In pure oxygen the rate of urine formation was possibly very slightly increased over that in room air, but it is not certain that this increase was significant. The main object of the measurements was to show that the change was by no means directly proportional to the difference of the oxygen tensions. In low tensions of oxygen urine formation was very definitely inhibited. It was previously proved that in the absence of oxygen

no urine formed, and there were no exceptions to this in the large number of frogs studied. In concentrations of oxygen between zero and room air, however, the results were variable. In the same concentration and in the same series of frogs, some frogs formed no urine, while others formed practically the normal amount of urine. This result was not at all due to differences in breathing, since in 4 per cent oxygen, for instance, breathing and muscle tonus were maintained in all animals. The average values for the rate of urine formation shown in figure 4 were rarely reproduced by any one frog and represent a statistical result.

The urine formation in frogs whose brains were destroyed without further operation was described in some detail in another paper (Adolph, 1934b); these frogs were studied in the presence of room air. When pithed frogs were subjected to pure oxygen, much more urine was formed, although this amount of urine fell short of that observed in normal frogs and was also less than that observed in frogs that were not only pithed but also operated upon so that the viscera were exposed. Obviously in the operated frogs the greater exposure of tissues allowed better aeration of the blood. The relationships found for pithed frogs would of course be quantitatively different at lower temperatures, but on the whole the rather high temperatures employed emphasized the differences between pithed frogs and normal frogs. In the pithed frogs there was little chance of recovery in rate of urine formation, because once breathing had ceased it could not be resumed, due to the injury to the nervous system. In contrast, the breathing of normal frogs might be stopped by asphyxia, only to be resumed as soon as they were given an atmosphere containing some oxygen.

COMMENT. In the normal frog the rate of urine formation was modified markedly only in tensions of oxygen less than those of room air. In these tensions the blood was obviously incompletely oxygenated and so the normal amount of oxygen did not get to the kidneys. In pure oxygen the normal frog, of course, had little more oxygen in its blood than it did in room air. Whether this was the reason why no more urine formed in oxygen than in room air is not certain, for it might also be that in room air the renal vessels or the renal tissues already had all the oxygen that could influence them.

The pithed frog was affected to such a marked degree by the oxygen tension prevailing because of the circumstance that the breathing was interrupted and the supply of oxygen to the blood was very poor, even in an atmosphere of pure oxygen. The pithed frog obtains all its oxygen by diffusion through the skin and other exposed surfaces, and so it was not surprising that the rate of urine formation was proportional to the oxygen tension between the limits of 0 and 100 per cent of an atmosphere. This fact does not necessarily imply, however, that oxygen was required by the renal tissues in order that water be excreted. Apparently the renal arterioles responded, at least statistically, to the oxygen pressure in the

blood coursing through them; only upon this view can the changes of blood flow and of urine production be assigned to a common mechanism.

In a great many experiments done in the past, in which urine formation was measured in frogs that were pithed or otherwise rendered non-breathing, it is evident that maximum rates of urine production could not have been observed so long as room air and ordinary temperatures prevailed. This factor of partial asphyxia must be of great importance in attempting to induce diuresis, for instance. Either an expected diuresis would not occur because of the condition of the glomerular circulation, or an abnormal diuresis would occur in contrast to an already reduced circulation which might not occur in contrast to an adequate circulation. Again, in the collection of fluid from the capsules of the kidney such as has been carried out very extensively by Richards (1929), maximum quantities of fluid are desired. If the amount of urine be proportionate to the amount of glomerular fluid and vice versa, then it is obvious that glomerular fluid production can be greatly enhanced in the manner that has now been described for urine production.

The mechanism by which the renal arterioles control the amount of urine produced is not fully elucidated, but it is evident that the systemic circulation is by no means embarrassed in proportion to the glomerular circulation. Hayman (1927) measured the diastolic pressure within the glomerular capillaries of pithed frogs, and found it to be on the average 20 cm. of water. If it be assumed that this pressure is the force responsible for the formation of urine, then so long as it exceeds 11 cm. of water, which is the usual colloid osmotic pressure of the plasma (White, 1924), urine might be expected to form. In the measurements of Hayman, the glomerular capillary pressure tended to be proportional to the aortic arterial pressure. In the present observations the aortic pressure may have occasionally decreased to 55 per cent of its normal value in the complete absence of oxygen (fig. 3), but not always. In room air there was no decrease of arterial pressure compared with that in pure oxygen. Hence if urine production is due to hydrostatic forces, the afferent renal arterioles, in low oxygen tensions, appear to have the effect of cutting off the glomeruli from the large arteries, thereby reducing the pressure in the glomerular capillaries.

Instances are at hand in which the number of glomeruli conducting blood was as great in the low oxygen tensions as in the high, and yet the rate of urine production was only one-tenth of that in the high oxygen tensions. Evidently the lack of blood flow through the glomeruli is one limiting factor in urine production, while the presence of rapid blood flow does not insure urine formation. In both these respects the effect of low oxygen tension is exactly comparable to the effect of high carbon dioxide tension (Adolph, 1935).

SUMMARY

1. The pithed frog produces urine at a rate that diminishes sharply with the oxygen tension that prevails. Modifications of the glomerular blood flow, of the mean aortic pressure, and of the heart rate, are not proportional to wafer excretion.

2. The normal frog, by virtue of its breathing, maintains its rate of urine formation nearly independent of oxygen tensions above the tension of room air. In lower tensions the response varies in individuals and on the average declines rapidly with oxygen tension.

3. The response of the renal arterioles that reduces the blood flow and probably also the capillary pressure in the glomeruli appears to be under the direct influence of oxygen tension. It is important that kidney experiments on the operated frog be conducted under high oxygen tension.

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THE MANNER IN WHICH THE ELECTRIC CURRENTS GENERATED BY THE HEART ARE CONDUCTED AWAY

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The problem of explaining the production of electrical deflections at a distance from the heart, such as occur in the standard electrocardiogram has been and still is an intriguing one. Many of the controversies which have arisen have had their origin in the attempt to account for the entire process without adequately considering all aspects of the problem or erroneously dismissing some as of no importance (cf. Katz, 1). The concept that the electrical field created by the heart obeys with but little modification, the rules of a finite spherical field has been implicitly accepted (cf. Craib, 2; Wilson, MacLeod and Barker, 3). Only recently has the work of Eyster, Maresh and Krasno (4) shown that the electrical field in reality approximates more nearly that of a plane and they have shown that it is not homogeneous. Actual experience (5), (6) as well as mathematical (7) and other considerations (4) have shown that the Einthoven triangle is at most only a rough approximation; in fact the question has arisen whether the Einthoven triangle concept is tenable. The criticism offered by Wilson and his colleagues of Zeisler's deduction evades the issue that the equilateral triangle can not be derived from trigonometric considerations without many unjustified assumptions.

The consideration paid to the Einthoven triangle concept has diverted the attention from the actual pathways taken by the currents generated by the heart. The electrical conductivity of the various tissues in the body is of considerable importance in the development of the electrical field, as has been recognized and worked out in part by Eyster et al. (4). It has not been fully appreciated, however, that the relative electrical conductivity of the tissues immediately adjacent to the heart is of the utmost importance in this regard. This is so for several reasons: 1, the pathways taken by the electric currents to the rest of the body will be determined by the relative conductivity of the structures surrounding the heart, thus greatly influencing the electrical field; 2, regions of the heart adjacent to tissues of

[1] Aided by the Frederick K. Babson Fund for the Study of Diseases of the Heart and Circulation, Michael Reese Hospital.

relatively good conductivity will exert a much greater influence on the deflections obtained from indirect leads than those adjacent to relatively poor conductors and this influence of a given cardiac region will be more or less a function of the relative conductivity of the body tissue adjacent to it.

During the course of experiments in which ventricular volume curves were recorded simultaneously with the standard three leads of the electrocardiograph, it was found that the use of a glass-rubber oncometer to enclose the ventricles decreased the amplitude of the electrical complex of the ventricle to such a degree that in some instances it was barely detectable, and in others it became reduced to the magnitude of the electrical complex of the auricle which had remained practically unaltered.[2] The oncometer obviously had insulated the electric currents in the ventricles except for the pathways offered by the systemic and pulmonary blood vessels, the auricles and their contained blood, which thus appeared to be poor conductors. This observation was so surprising that we decided to investigate the relative importance as electrical conductors of the various structures in contact with the heart. Our approach was by the experimental method and we have refrained from using a priori mathematical reasoning which, because of the inability to take into account all possible variables, has been, at least partially, misleading in the past.

PROCEDURE. Dogs under morphine and barbital anesthesia were employed. Most of the animals were placed on their backs, but in a number of experiments the animals were placed in other positions. In all the chest was opened and artificial respiration was used. The usual three leads of the electrocardiogram were obtained in the customary fashion from limb leads using German silver electrodes surrounded by gauze soaked in a concentrated salt solution. These were placed subcutaneously.

In the 8 dogs placed on their backs, the entire anterior chest wall was removed. In the 4 dogs placed on their right or left sides, the opening was in the opposite chest wall. In the 2 dogs placed on their abdomens, the opening was in the left side of the chest. The opening in the lateral chest wall was made by removing two ribs for a distance of 4 inches and the opening enlarged with retractors. The intact pericardium was sewed to the chest wall with stay sutures to immobilize the position of the heart as much as possible. Electrical insulation of the heart from the structures adjacent to it was obtained by the insertion of rubber sheeting 1 mm. in thickness and of appropriate size in certain positions.

An electrocardiogram was obtained in each dog before and immediately after the chest was opened and the sutures placed. An electrocardiogram was taken during each type of insulation, using as controls the electro-

[2] When discussing this subject with H. B. Williams we were informed that he and J. S. Wheelright made a similar observation in 1911–12, which was not published.

TABLE 1

The effect of insulating the heart on the sum of the major deflections of QRS in the three standard leads

DOG NUMBER	(A) MASSIVE INSULATION OF HEART EXCEPT FOR SYSTEMIC AND PULMONARY VESSELS	(B) INSULATION OF HEART FROM ANTERIOR CHEST WALL	(C) INSULATION OF HEART FROM POSTERIOR CHEST WALL	(D) INSULATION OF HEART FROM DIAPHRAGM	(E) INSULATION OF HEART FROM RIGHT LATERAL CHEST WALL	(F) INSULATION OF HEART FROM LEFT LATERAL CHEST WALL	(G) INSULATION OF LUNGS FROM HEART AND ENTIRE CHEST WALL EXCEPT FOR PULMONARY VESSELS BETWEEN FORMER TWO	(H) INSULATION OF HEART FROM SYSTEMIC VESSELS
			Dog on back					
1	++++		++++					±
2	++++	±	+++	++				
3	++++		+++	++			±	±
4			++++				±	±
5	++++	±	+++	±			±	
6	++++		++++	±			+	±
7	+++	±	++	±			±	
8	++++		+++				±	
			Dog on abdomen					
9	++++	+++	±					
10	+++	++	±	±				
			Dog on left side					
11	+++	±	±	++		++		
12	++++	±	±	+		++		
			Dog on right side					
13	++++	±	+++	++	+			
14	+++	±	+++	+++	±			

± is equivalent to decrease of from 0 to 15 per cent.
+ is equivalent to decrease of from 15 to 25 per cent.
++ is equivalent to decrease of from 25 to 50 per cent.
+++ is equivalent to decrease of from 50 to 75 per cent.
++++ is equivalent to decrease of from 75 to 100 per cent.

Note: The sums of these percentages, because of the roughness of the approximation, of course do not give 100 per cent.

cardiogram obtained immediately before the insulator was in place and the one taken immediately after removal of the insulator.

The following types of electrical insulation of the heart were tested:

a. Massive insulation, where the entire heart was electrically insulated except for the contacts via the large systemic and pulmonary blood vessels.

b. Insulation of the heart from the anterior chest wall.

c. Insulation of the heart from the posterior chest wall.

d. Insulation of the heart from the diaphragmatic wall.

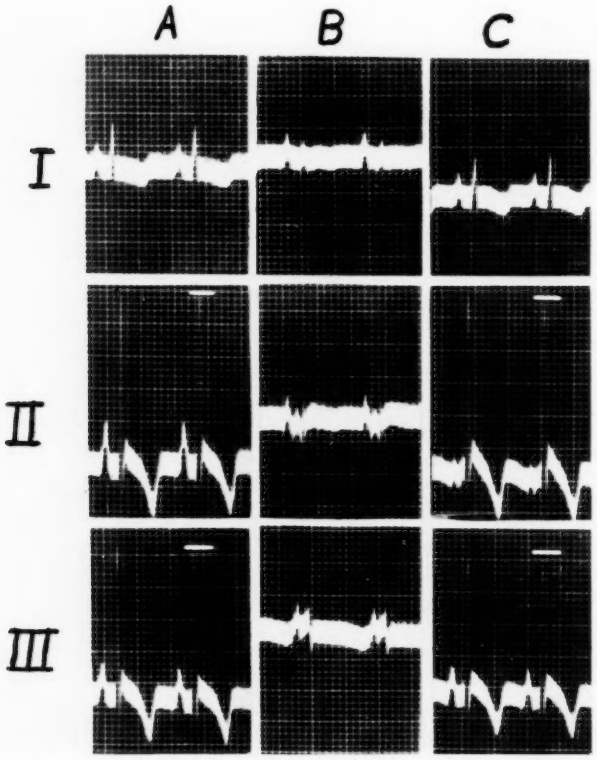

Fig. 1. Segments of the conventional three lead electrocardiograms from experiment 6 with dog on its back to show the striking reduction in the amplitude of the ventricular complex (QRST) following insulation of the heart from the posterior muscle mass. The change would be of the order described in table I as ++++. Segment A is the control before and segment C, the control after the insulator had been used. Segment B is the curve obtained with the insulator in place. Time 0.04 and 0.2 segments, ordinates 1 and 5 millivolts. Heavy white lines in leads II and III of segments A and C show height of major deflection of QRS complex.

e. Insulation of the heart from the right lateral chest wall.

f. Insulation of the heart from the left lateral chest wall.

g. Insulation of the lungs—in which the lungs were insulated from the chest walls and the heart except for the contact between heart and lungs via the pulmonary vessels.

h. Insulation of the heart from the systemic blood vessels, for which purpose certain vessels were ligated and cut, namely, the arteries arising from the arch of the aorta, the descending aorta, the superior and inferior vena cava and the azygos vein.

RESULTS. Although many alterations in contour of the various electrocardiographic deflections occurred and shifts in the electrical axis were encountered, we confined our attention to a relatively simple procedure in measuring the effects of the various types of insulation. It consisted of determining in millivolts the sum of the heights of the major deflection of the QRS complex in the three leads of each record; the direction of the deflections was disregarded in obtaining this sum. The sum of the heights so obtained was compared with the sums of the heights similarly obtained in the control records. This rough method of quantitating the results we found sufficiently accurate for our purpose.

The results are shown in table 1 where the degree of change caused by the insulation is expressed as ±, +, ++, +++ and ++++, depending on the percentage change from the control reading (see footnote of table). In figure 1 is shown the effect of insulating the heart from the posterior muscle mass in experiment 6 to illustrate the magnitude of the change designated by us as ++++.

It will be seen from this table that the electric currents generated by the heart are not conducted away with equal facility by all parts of the adjacent structures. Thus when the animal is lying on its back with chest open, most of the current is conducted away by the posterior muscle mass. Insulation between the heart and this region is practically as effective as massive insulation. In two animals the diaphragm carried away some of the current, but in the rest it was found to be no more important than the lungs, anterior chest wall and the systemic vessels which carried away little of the currents of the heart.

The relative conductivity of the various structures was altered considerably when the animal was placed in other positions. Thus it was found that with the animal on its abdomen, the anterior chest wall became the important conductor electrically, the posterior chest wall and diaphragm becoming relatively unimportant. The left lateral chest wall and the diaphragm were the most important conductors when the animal was on its left side while, surprisingly enough, the posterior muscle mass and diaphragm were the important conductors when the animal was on its right side, the right lateral chest wall being relatively unimportant.

DISCUSSION. The explanation for these results is not difficult to find. The lungs, while intimately in contact with the heart, are poor electrical conductors, more so apparently than has generally been appreciated. The fact that lungs consist of such a fine lattice work of conducting tissue enmeshing a dielectric material, the alveolar air, probably accounts for their poor conducting ability. It is more surprising that the large systemic blood vessels were such poor conductors, particularly in view of the impression given by early workers that the vascular system filled with blood is an excellent conductor.

Our work shows clearly that the solid masses of muscle are the best electrical conductors but, to be effective, they must be in intimate contact with the heart. Variation in the intimacy of contact of the heart in the different positions of the animal explains why different muscle masses assume the predominant rôle as electrical conductors. The mobility of the dog's heart with change in the dog's position is easily ascertained fluoroscopically or by inspection when an opening is made in the chest.

It is this mobility of the canine heart which explains the importance of the posterior muscle mass as an electrical conductor when the animal is on its back. The importance of the diaphragm will depend on its position as determined by the degree of abdominal distention, and the variation in its position probably accounts for the varying amount of currents carried off by the diaphragm in different experiments when the animal is on its back. The mobile canine heart shifts when the animal is on its abdomen, comes in contact with the anterior chest wall, and moves away from the posterior muscle mass with the result that the anterior chest wall now carries off practically all the current. When the animal is on its right side, the presence of the lung between the heart and right chest prevents this region from conducting off much of the current. Instead the posterior chest wall and diaphragm conduct away most of the current because the heart comes in direct contact with these regions with no lung tissue interposed. When the animal is on its left side there is a region between the heart and antero-lateral chest wall where no lung intervenes. The heart consequently comes in intimate contact with this region as well as with the diaphragm. Both of these regions now become the important electrical conductors carrying the heart currents to the rest of the body.

These results are strongly indicative of the fact that many of the changes in the electrocardiogram with changes of body position formerly ascribed to shifts in the electrical axis of the heart, are in the dog and probably also in man to be attributed to 1, changes in the important conduction pathways adjacent to the heart, and 2, to changes in the regions of the heart in contact with these good conductors. It follows from this that the terms "electrical axis" and "shift in electrical axis" are, strictly speaking, misnomers, since they give the impression that all regions of the heart con-

tribute approximately equally in giving rise to an electrical vector and that changes in the field are due to reorientation in three dimensions of this resultant vector. The alternative viewpoint which our results suggest tends to minimize the classical vector analysis and emphasizes the importance of analyzing the relationship of the various regions of the heart with good and poor electrical conductors in interpreting changes in the electrocardiogram.

The human chest compared with the dog's is wider laterally, shorter from neck to diaphragm and shallower antero-posteriorly. The human heart is therefore more intimately in contact with the chest than the canine heart: this we have readily verified by post-mortem examination of man and the dog. It is these anatomical facts that make the human heart less mobile than the dog's heart.[3] There is therefore probably less variation in the conduction pathways in man with change of body position, although alterations in the position of the various parts of the heart in relation to various regions of the chest will still occur. Postmortem examination by us suggests that the regions of good electrical conduction, where heart and parietes are in intimate contact without lung tissue intervening are: 1, the lower left antero-lateral chest wall—the region of absolute precordial dulness; 2, the diaphragm, and 3, the lower portion of the posterior muscle mass surrounding the vertebral column. The anterior chest wall is in contact with the right ventricle and a small rim of the left ventricle adjacent to the interventricular groove. The posterior muscle mass is in contact with the left ventricle. The diaphragm shares its contact equally with right and left ventricles, the former contact being anterior to the latter and separated from it by the interventricular groove.

Variations in the relation between different parts of the heart and the good and poor conductors of the surrounding structures are readily produced by changes in position of the heart, with hypertrophy, dilatation of the cardiac chambers and by displacements of other origins. Our work suggests that these altered relationships explain many of the aberrations observed in the electrocardiogram in both healthy and diseased hearts. These aberrations in the electrocardiogram are not to be regarded as alterations in the orientation of the electrical vector, the manifest potential, but rather, so to speak, in the orientation of silent and non-silent regions of the heart determined by the location of the various regions as regards the good and poor electrical conductors. The use of precordial leads is in reality an attempt to explore some of these silent regions of the heart by getting nearer to the current source and not, as some believe, the establishment of the third dimension of the so-called electrical vector.

From this brief discussion it will be clear that the results reported by us

[3] The denser nature of the mediastinum in man also immobilizes the heart somewhat.

put a different perspective on the meaning of the electrical field of the heart than that generally held. It opens up the entire subject of interpretation of the electrocardiogram on a newer and simpler basis. Less attention need be paid to the calculation of vector angles and computations of the properties of the so-called Einthoven triangle and more attention must be directed to a study of the position changes of the various parts of the heart under diverse circumstances.

SUMMARY

1. Experiments were performed to determine the paths taken by the currents generated by the heart in different positions of the body.

2. It was found that there was a striking disparity in the electrical conductivity of the structures adjacent to the heart. The lungs and the large systemic and pulmonary vessels coming off from the heart were found to be poor conductors, while the muscular structure of the chest wall, especially the diaphragm and posterior muscle mass, were found to be good conductors.

3. The paths by which the heart's currents were carried away were altered in different body positions due to the mobility of the heart. This is discussed in detail. Evidence is given to show that these data can be applied to man.

4. Aberrations of the electrocardiogram, on the basis of this study, are to be regarded as the result largely of reorientation of the various regions of the heart with good and poor electrical conductors or to changes in the important conduction pathways adjacent to the heart.

5. This work again calls into question the accuracy, even as a first approximation, of the modern versions of the Einthoven equilateral triangle concept. The interpretation of electrocardiograms on the basis of the paths actually taken by the electric currents, especially from the heart to adjacent structures, offers a simpler and more practical concept.

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MAINTENANCE OF THE LIGHT REFLEX AFTER DESTRUCTION OF THE SUPERIOR COLLICULUS IN THE CAT[1]

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Largely perhaps because its actual course has been unknown, the afferent path of the pupillo-constrictor reflex in response to light has long been held to traverse the superior colliculus in its passage to the oculomotor nucleus. Opposition to this view has been advanced by a number of investigators, however,—Knoll (1), Bechterew (2), Bernheimer (3), Levinsohn (4), and Keller and Stewart (5)—who found the light reflex to remain intact after destruction of the anterior quadrigeminal body.

In a series of experiments in which the diencephalon and midbrain of the cat were electrically stimulated with the aid of the Horsley-Clarke stereotaxic instrument, the extent of the course of the light reflex pathway was recently traced by Ranson and Magoun (6). It was clear from these experiments that the light reaction of the pupil was mediated by the pretectal region, or transition zone between thalamus and midbrain, and not by the superior colliculus. In a second investigation, the results of this study were checked by observing the effect upon the light reflex of lesions of the pretectal region (7). It was found that the amount of reduction in the light reflex varied directly with the amount of pretectal destruction, and abolition of all but a trace of light reaction was achieved by lesions confined entirely to the pretectal region. Larger lesions of this region which led to a total loss of the reactivity of the pupil to light, injured also the rostral portion of the superior colliculus.

In an effort to rule out completely the participation of the superior colliculus in the light reflex, it was decided to produce lesions of the superior colliculus, and then to see whether any impairment in the light reactions of the pupil could be noted. The results of this study, which indicate that destruction of the superior colliculus leads to no impairment of the light reflex so long as the pretectal region remains uninjured, form the subject of the present report.

METHOD. Lesions of the superior colliculus were produced in six cats with the aid of the Horsley-Clarke stereotaxic instrument, the detailed use of which has been reported elsewhere (8). This instrument may be briefly

[1] This investigation was aided by a grant from the Rockefeller Foundation.

described as an apparatus adjustable to the animal's head which enables a needle-like electrode, insulated except at the tip, to be accurately oriented within the interior of the intact brain.

Destruction of the superior colliculus in previous investigations has entailed as a rule rather gross section or retraction of the overlying brain parts. The advantage of the use of the Horsley-Clarke instrument in producing localized injury to this region may be indicated by the fact that with this technique the approach may be made through an opening of about one-half square inch in the calvarium, the meninges and cerebral hemispheres overlying the colliculus remaining intact, except for the minute electrode punctures through them.

In producing a lesion of the superior colliculus, all aseptic precautions were taken. Under Nembutal anesthesia, the unipolar electrode was inserted into the brain $1\frac{1}{2}$ mm. to the right and left of the midline in a plane 4 mm. anterior to the zero point of the instrument, and $1\frac{1}{2}$ and $2\frac{1}{2}$ mm. to the right and left of the midline in planes 3 mm. and 2 mm. anterior to the zero point of the instrument. On each of these punctures lesions were produced at points $4\frac{1}{2}$, $3\frac{1}{2}$, and $2\frac{1}{2}$ mm. dorsal to the zero point of the instrument. In one animal of the series, cat B, the lesions were produced 0.5 mm. further rostrally, in planes 4.5, 3.5, and 2.5 mm. anterior to the zero point of the instrument. Each lesion was produced by the electrolytic effect of the passage of a direct current of 3 milliamperes for 30 seconds, the indifferent electrode being in the mouth.

The nictitating membranes were removed to permit unhampered examination of the pupils, and following operation the light reactions of the animal were usually tested each day for the first week and two or three times a week subsequently until the sacrifice of the animal five or six weeks after operation. In testing the light reactions, the animals were examined while resting in a hammock in a dimly lighted room, and the width of the pupils was measured in millimeters before and during illumination, both for the direct and consensual reactions. A fountain pen flashlight with a focussed bulb was employed in lighting the eye, new batteries being substituted at short intervals.

At sacrifice the brain was injected in situ with formalin; drawings were made in each case of the position of the lesion on the dorsal aspect of the brain stem, and in two cases photographs were made. A block including the lesion was embedded, cut in serial transverse sections, and every fourth section through the lesion was stained by the Weil method and mounted. The extent of destruction was then determined by microscopical examination.

Results. In studying the light reactions of the six cats in which lesions had been placed in the superior colliculus, it became apparent that in two cats the light reflex remained wholly unimpaired and normal, in three cats

the light reactions were somewhat reduced, and in one cat the reactivity of the pupils to light was completely abolished. It would immediately appear that while all of these lesions were adjacent to that portion of the brain stem which mediates the pupillary light reflex, this region remained intact in two cases, in three cases it was partially injured, and in one case it was completely destroyed.

The series of observations on one of the animals in which the light reactions remained unimpaired (cat A) and those on the single animal in which the light reactions were completely abolished (cat B), may each be reported in detail, since these are of greater interest than the others. The case in which the light reactions remained unimpaired may first be considered. Cat A (table 1) exhibited light reactions of a normal excursion throughout the five weeks that it was allowed to live after operation. On lighting either eye, direct and consensual pupillo-constriction to a slit measuring

TABLE 1

Cat A. Operated June 15

DATE	BEFORE LIGHTING		LIGHTING RIGHT EYE		LIGHTING LEFT EYE	
	Width right pupil	Width left pupil	Width right pupil	Width left pupil	Width left pupil	Width right pupil
June 16	1.5	1	0.5		0.5	
June 18	5	6	1	1	1	1
June 19	5	7	0.5	1	0.5	0.5
June 21	7	8	0.5	0.5	0.5	0.5
June 28	7.5	8	1	1	1	1
July 23	10	9	1	0.5	0.5	1

All measurements in millimeters.

1 mm. or less in width, ensued. From a quantitative point of view, therefore, the light reflex of this animal remained normal and unimpaired.

The lesion (figs. 1A and 2) destroyed the medial two-thirds of the superior colliculus throughout its rostrocaudal extent and reached ventrally to the dorsal border of the cerebral aqueduct. The injury disappeared rostrally at the transition to the pretectal region and terminated caudally at the rostral border of the inferior colliculus. The pretectal region together with the superior quadrigeminal brachium and its connections with the lateral third of the superior colliculus remained intact.

While the light reactions of this animal (cat A) remained quantitatively normal, two disturbances of pupillary innervation were, however, apparent. On the day after operation the pupils were markedly miotic (in a dimly lighted room), measuring right 1.5 mm. and left 1 mm. in width, and four days after operation slight miotic tendencies were still present on the right side, the pupils measuring right 5 mm. and left 7 mm. For the

remainder of the survival time the widths of the pupils varied between 7 and 10 mm. which may be considered normal.

The second abnormality in the pupillary behavior of this animal could be detected after the light reaction had taken place. On cessation of illumination of the eye, the pupils dilated very slowly, instead of quickly regaining their original width as in the normal animal. During the first

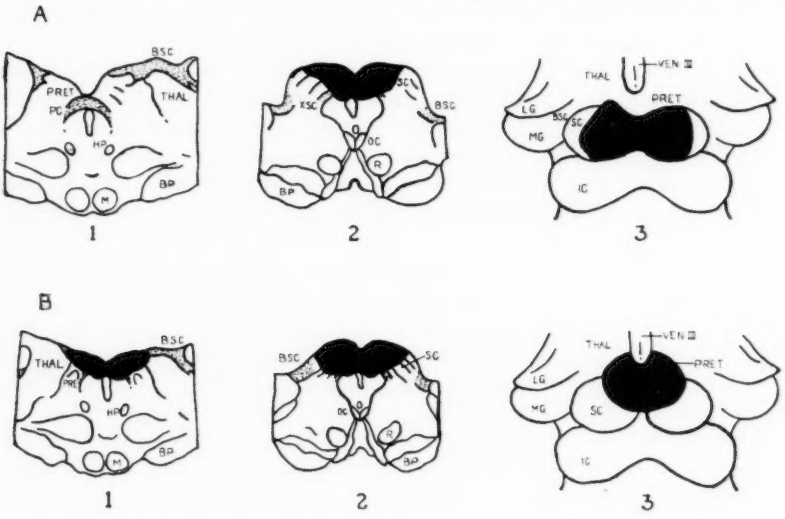

Fig. 1A. Projection drawings of transverse sections through the pretectal region (1) and superior colliculus (2) of cat A, and a diagrammatic view of the dorsal aspect of the brain stem of the cat (3). The extent of the lesion in cat A is shown in solid black.

Fig. 1B. Projection drawings of transverse sections through the pretectal region (1) and superior colliculus (2) of cat B, and a diagrammatic view of the dorsal aspect of the brain stem of the cat (3). The extent of the lesion in cat B is shown in solid black.

Abbreviations for all figures:

BP, basis pedunculi; *BSC*, brachium of the superior colliculus; *HP*, habenulopeduncular tract; *IC*, inferior colliculus; *LG*, lateral geniculate body; *M*, mammillary body; *MG*, medial geniculate body; *OC*, oculomotor nucleus; *PC*, posterior commissure; *PRET*, pretectal region; *R*, red nucleus; *SC*, superior colliculus; *THAL*, thalamus; *VEN III*, third ventricle; *XSC*, commissure of the superior colliculus.

two weeks after operation this slow dilatation of the pupils after illumination was not materially hastened by painful stimulation. Five weeks after operation, however, the pupils briskly dilated after illumination, when the animal was painfully stimulated, but when not interfered with, the pupils still required a longer time than was normal to dilate to their original size.

As was reported in a previous communication (7), a transitory miosis and an after-constriction of the pupil on cessation of illumination of the eye both follow lesions in the caudal portion of the thalamus. After such thalamic lesions the pupillary light reactions suffer a transitory impairment but then become quantitatively normal.

The effects of a lesion in the superior colliculus (cat A), therefore, closely resemble those of a lesion in the caudal portion of the thalamus. After each the light reactions are quantitatively normal, but an after-constriction of the pupil following cessation of illumination of the eye is apparent, and a transient miosis of the pupil may be observed. Since the light reactions of the pupil remain of normal extent in each case, it is impossible to believe that the pupillary after-constriction and miosis are due to a direct involvement of the light reflex pathway by these lesions. The most reasonable explanation appears to be that the influence of these lesions on pupillary

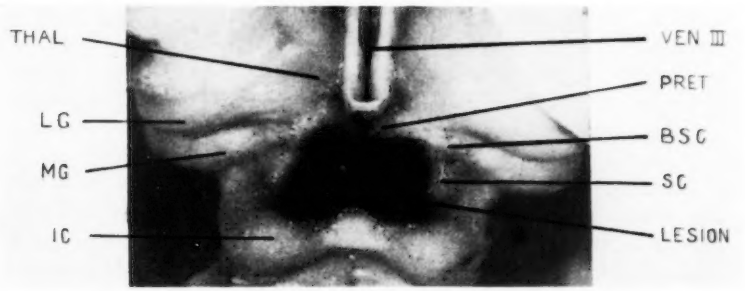

Fig. 2. Photograph (reproduced about twice natural size) of the dorsal aspect of the brain stem of cat A. The lesion is seen in the superior colliculus.

innervation is effected indirectly, either through irritation or some other factor, on that region of the brain stem to which both the thalamus and the superior colliculus are adjacent, namely, upon the pretectal region, which is situated between them.

The essential rôle of the pretectal region in the sphincter innervation of the pupil has already been indicated (6), (7) and confirmatory evidence for this view is furnished by the observations made on cat B which may now be reported.

During the first week after operation cat B (table 2) exhibited a possible trace of pupillo-constriction in response to light, but because of the presence of hippus, it is questionable whether any light reaction actually existed. For the remaining five weeks of survival of this animal, no reaction to light could be detected on repeated testing. Two other disturbances of pupillary innervation were present and often interfered with examinations of

the light reactions. The first of these was the hippus already mentioned, which persisted for the survival time of the animal. The second was a pupillo-constriction associated with a retraction of the eyeball and attempted lid closure, which could be induced in part by simply holding the lids open, without any change in the illumination of the eye. The pupils could then be further constricted by irritating the palpebral conjunctiva near the inner canthus, thus causing vigorous retraction of the bulb and attempts to close the lids (9). On the day following operation such a pupillo-constriction from 2 mm. to a slit was present. Two weeks after operation a pupillo-constriction from 11 mm. to 8 mm. could be induced by this method, and six weeks after operation a pupillo-constriction from 8 mm. to 3 mm. could be so produced. A transitory miosis was apparent on the day after operation when the pupils measured 2 mm. in width, but not subsequently when the width of the pupils varied between 8 and 11 mm.

The lesion (fig. 1B) was found to extend from the caudal portion of the thalamus to the middle of the superior colliculus. Both habenulae and the adjacent portion of the nucleus lateralis posterior were softened. Both halves of the pretectal region were symmetrically destroyed from the medial edge of their brachial connections to the midline and ventrally to the central grey matter immediately below the posterior commissure. The damage to the superior colliculus was confined to its rostral half, the medial two-thirds of each side of the colliculus being destroyed down to the dorsal border of the cerebral aqueduct. The caudal half of the superior colliculus remained wholly intact, as did the superior quadrigeminal brachium and its connections with the lateral third of the superior colliculus rostrally.

In order to determine what part of the brain stem is concerned in mediating the pupillary light reflex, the results from cats A and B may now be compared and a correlation made between the extent of the lesion and its effect upon the light reflex in each case. In cat A, in which the light reflex remained normal, the medial two-thirds of the extent of the superior colliculus was destroyed, while the caudal part of the thalamus, the pretectal region, and the lateral third of the superior colliculus remained intact. Since the light reflex remained unimpaired after its destruction, the extent of the medial two-thirds of the superior colliculus may immediately be eliminated from further consideration. The maintenance of the light reaction in this animal (cat A) may be ascribed to the retention either of the caudal portion of the thalamus, the pretectal region, or the lateral third of the superior colliculus, each of which remained intact.

In cat B, in which the light reflex was abolished, the caudal portion of the thalamus and the pretectal region were destroyed, while the lateral third of the superior colliculus remained intact. Since the light reflex was

abolished while this region remained uninjured, the lateral third of the superior colliculus can also be eliminated from consideration, and the suggestion of Spiegel and Nagasaka (10) that the light reflex is mediated by the lateral portion of the superior colliculus can no longer be held. Since it has already been demonstrated that a normal light reflex exists after destruction of the caudal portion of the thalamus (7), the conclusion is inescapable that the abolition of the light reflex in the present animal (cat B) was due to the destruction of the pretectal region. Conversely, the maintenance of the light reflex in cat A can be ascribed to the retention of the pretectal region in that animal. It may also be concluded, without qualification, that no part of the superior colliculus is concerned in mediating the pupillo-constrictor reflex in response to light.

The three animals in this series in which the light reactions were somewhat reduced need not be considered in detail since the results which they

TABLE 2

Cat B. Operated June 7

DATE	BEFORE LIGHTING		LIGHTING RIGHT EYE		LIGHTING LEFT EYE	
	Width right pupil	Width left pupil	Width right pupil	Width left pupil	Width left pupil	Width right pupil
June 8	2	2	Pupils slit-like on holding lids open			
June 12	9	9	8	8	7.5 to 9	7.5
June 15	11	11	No reaction		No reaction	
June 28	10	10	No reaction		No reaction	
July 17	10	10	No reaction		No reaction	
July 24	8	8	No reaction		No reaction	

All measurements in millimeters.

illustrate have already been presented at some length (7). These results may be briefly summarized by stating that partial injury to the pretectal region leads to a reduction in the light reflex, and if this injury is unilateral, the light reactions of the contralateral eye are the more severely impaired.

SUMMARY

1. After widespread destruction of the superior colliculus in the cat, the pupillary reactions to light remain normal and unimpaired.

2. With coincident destruction of the pretectal region, the pupillary light reactions are completely abolished. The pretectal region contains an essential part of the light reflex arc.

3. No part of the superior colliculus is concerned in mediating the pupillary light reflex.

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THE RELATION OF RED CELL DIAMETER AND NUMBER TO THE LIGHT TRANSMISSION OF SUSPENSIONS

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Oliver (1895–96) was the first to describe a method whereby the number of red cells per cubic millimeter of blood can be found by measuring the opacity of a suspension, the method consisting in observing a candle flame through a flattened tube containing Hayem's solution, and adding blood until the flame cannot be seen. The method does not appear to have been much used, and Holker (1921) later described a very similar method in which the "opacity" (strictly speaking, the density[1]) of the suspension is measured by viewing a wire, lit from behind, through a column of suspension of variable length. Holker claims great accuracy for his method, and concludes that the number of red cells in a suspension can be accurately determined from the opacity, which also gives, by the use of a simple equation, the average diameter of the cells, provided the number present is known. Jacobs (1930) comments on the relation between number and opacity, and suggests the use of opacimetry as a method of red cell counting; all the other investigations in the literature seem to be concerned with the mathematical expression of this relation, and not with the use to which opacimetric methods might be put (Vlés, 1919; Cheveneau and Audubert, 1919; Achard, 1931, 1932; see also Houghton, 1931; Stratton and Houghton, 1931; Ryde and Cooper, 1931; Watson and Kibler, 1931; and Sauer, 1931, as examples from the physical literature). Taken together, these investigations show that the opacity of suspensions is not susceptible to a simple theoretical treatment, and so I shall confine myself to a purely experimental examination of the adequacy of the opacimetric method when applied to red cell counting and the determination of red cell diameters.

METHODS. All the measurements of opacity were made with the Pulfrich Stufenphotometer in conjunction with the water bath and glass

[1] In Holker's method the results are given in terms of the scale readings of his apparatus, and these, according to Lambert's law, are linear with the logarithm of the light transmitted, i.e., are measurements of *density*. Opacity is the ratio of the light entering the suspension to that transmitted through it. Jacobs' method also measures density directly on the scale.

chambers already described (Ponder, 1930). The suspensions were contained in a glass chamber of a little more than 2 cc. capacity and 5 mm. thick, and the light used was that supplied by the green L2 filter belonging to the apparatus. This transmits light between 5100 A. U. and 5600 A. U., with maximum transmission at 5300 A. U., i.e., in the region of the oxyhemoglobin absorption bands.

Measurements were made on suspensions covering two arbitrarily selected ranges of concentration, the first variety being about as dilute as those used with Holker's apparatus, and the second being considerably more concentrated. In the case of the dilute suspensions, nearly all the light would be transmitted through the spaces between cells, while in the case of the concentrated ones nearly all of the light must have been scattered. To make the measurements 2 cc. of 1.0 per cent NaCl were placed in the chamber, and the transmission determined in terms of that through the "standard" chamber, similarly filled. Initial inequality can be corrected for. From a Thoma red cell diluting pipette, a volume of blood occupying 2 pipette divisions was added to the NaCl, stirred, and allowed to stand for 5 minutes in order to avoid the "variable light transmission" described by Kesten and Zucker (1928–29). A measurement of light transmission was made at the end of this time, and another volume of blood, this time occupying 2 pipette divisions, added. After another 5 minutes another measurement was made, and thereafter were added in succession first 2 pipette divisions and then 3 pipette divisions of blood, a measurement of transmission being made after each. In this way transmission measurements corresponding to volumes occupying 2, 5, 7, and 10 pipette divisions were obtained.[2]

The process was then repeated using more concentrated suspensions, the only difference being that a white cell diluting pipette was used, and that four successive volumes of 5 pipette divisions each were added to the fluid in the chamber, a transmission measurement being made after each. As these suspensions were relatively dense, the intensity of the "standard" beam was cut to 1 per cent of its initial intensity with a neutral filter.

The red cell counts for each animal were made by counting both sides of a Levy-Hauser hemocytometer, care being taken that the cells were properly distributed. Red cell diameters were found either photographically (Ponder and Millar, 1924), or diffractometrically (Ponder, 1933).

The transmission measurements are very reproducible, provided that "variable light transmission" is eliminated. If they are made immediately after stirring, there may be variations as great as 10 per cent.

[2] I have made no attempt to give results in anything but arbitrary units, because the numerical values depend on the dimensions of the chamber and the volumes delivered by the pipettes, etc., factors which it would be almost impossible to reproduce exactly, and also because the results cannot be reduced to a simple form, such as an extinction coefficient, for the theory underlying them is too complex.

Red cell counts. When the opacity for different concentrations of suspensions of human red cells is plotted against the number of cells per cubic millimeter of blood, graphs like those in figures 1 and 2 result, the former figure showing the values for dilute suspensions (in the sense explained above), and the latter the results for more concentrated suspensions. The smooth curves are drawn by the method of least squares. It will be seen that the scatter of the 40 points (4 points, corresponding to 4 different concentrations, for 10 samples of blood) is no greater when dense rather than dilute suspensions are used.[3]

Taking each point separately, and considering as an "error" the difference between the count corresponding to it (on the abscissa) and the count corresponding to a point on the smooth curve giving the same value for the

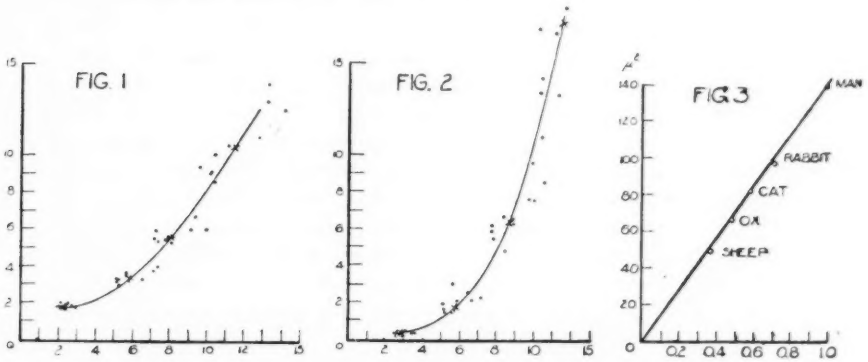

Fig. 1. Relation of opacity to red cell numbers for dilute suspensions of human erythrocytes. Ordinate, opacity; abscissa, numbers, both in arbitrary units. The curve passing through the crosses is the "best" relation between the variables.

Fig. 2. Similar to figure 1, but for dense suspensions.

Fig. 3. Relation of average values for red cell area to the value of the constant k.

opacity, we can express the "errors" as percentages in the usual way. They are far greater than those usually associated with hemocytometer counts, 54 out of the 80 being greater than ± 5 per cent, whereas the standard error of red cell counts made with the hemocytometer ought not to be greater than about ± 3 per cent (Ponder, 1934); the method is consequently a very inexact one if single readings are relied upon. Actually, however, four readings were made on each sample of blood, and if the results of the four are averaged, the errors become smaller. Thus in the

[3] The only advantage in working with dilute suspensions is that the number of cells is linear with the logarithm of the light transmitted. This is useful if theoretical expressions are being formulated, but does not add to the accuracy of the experimental procedure, which is greater when the suspensions are not very dilute.

case of the denser suspensions, they were −8, −8, −5, −4, −0.8, +2, +3, +6, +7, and +10 per cent, and a similar treatment of the data for dilute suspensions leads to a similar result. It is clear that if the method is to be used at all, several readings of light transmission ought to be made for different concentrations of cell suspension, and the results averaged in order to give individual errors the chance of cancelling out. Very similar results were obtained with blood of the rabbit, ox, and sheep.

Under any circumstances, the errors associated with the opacimetric method are apparently greater than those associated with the hemocytometer method, and there are two reasons why this should be so. 1. The red cell count as given by the hemocytometer, which is the standard with which the count found by opacimetry is compared, is not itself of absolute accuracy. Any difference between the "true" count and that found by the hemocytometer must accordingly be added (algebraically) to the difference between the figure given by the opacimeter and that given by the hemocytometer; sometimes the addition will lessen the difference, and sometimes it will increase it, but the general effect will be to make the opacimetric method appear less accurate than it really is. 2. Considering the individual values shown in figures 1 and 2, it is noticeable that the greatest discrepancies between the results of opacimetry and of direct counting are due to all of the points for some one sample of blood falling to the right or to the left of the smooth curve, i.e., that the greatest discrepancies are caused by systematic departures peculiar to that sample. The reason for this is obviously that the opacity of a suspension is a function not only of cell number, but also of cell size. This leads directly to the second part of the investigation.

The effect of cell size. I shall consider the results for the denser variety of suspension almost exclusively, as they are quite typical. If a series of "best" curves such as that shown in figure 2 is plotted for the blood of different animals (man, rabbit, cat, ox, and sheep), it will be found that the curves are all of a similar shape, but that the larger the cell size, the greater the value of the opacity corresponding to any given number. Apart from this, however, the curves are so similar that they can all be made to coincide with that for the blood of man by multiplying the numbers corresponding to successive opacities by a series of constants, whose values are: man, 1.00; rabbit, 0.72; cat, 0.59; ox, 0.49; sheep, 0.37. Considering, as Holker does, that the light-stopping power of the suspensions depends on the number of cells and on the individual cell area when projected on the plane of the faces of the chamber through which the light passes, these constants ought to be proportional to the average red cell areas. If the area is taken as $2.4\pi r^2$ (see Ponder, 1934, for how this approximation is arrived at), the proportionality is certainly striking (see fig. 3). This linear relation was predicted (and possibly obtained, but there are no data given) by Holker.

If projected surface area and number are the only two factors which determine the opacity, we ought to be able, as Holker claims is possible, to find the projected area, and thence the diameter of the cells, by measuring the opacity and making a red cell count. I have analysed my data in this way, and the following table shows a comparison between the figures so obtained and those which are found by direct measurement.

ANIMAL	DIAMETER, μ	
	Calc. from opacity	Direct measurement
Man 1	8.84	8.6
2	8.42	8.4
3	8.00	8.3
4	9.10	8.5
5	8.56	8.6
Rabbit 1	7.16	7.2
2	7.00	7.1
3	7.58	7.3
4	7.78	7.3
5	7.25	7.2
Ox 1	5.96	6.0
2	5.00	5.7
3	6.78	6.3
4	5.72	5.9

The agreement is not good, and the indirect method seems to give approximately correct results only when the cell diameter is close to the average value. This means that slight deviations from the smooth curves can be attributed to differences in cell diameter from the average for the species, but that the larger discrepancies have to be accounted for in another way. One of the most important reasons for their appearance seems to be that the opacity of red cell suspensions is dependent, not only on number and size, but on the shape of the cells.

The effect of cell shape and of the nature of the medium. The effects of differences in cell shape are illustrated in the following table, which gives the value of the constant by which successive values of opacity must be multiplied in order to make the curves coincide.

SUSPENSION MEDIUM	FORM OF CELLS	k
Plasma	Discs	1.00
Isotonic citrate	Discs	1.02
Isotonic NaCl	Crenated discs	0.94
Isotonic phosphate	Crenated discs	0.87
Plasma + lecithin	Spheres	0.80

The variation in the values of k show that changes in shape can produce considerable variations in opacity, and, since the amount of crenation in a cell suspension cannot be controlled, that the method is affected by an uncontrolled variable which may easily give rise to "errors" of the magnitude encountered.

The above results were obtained with dilute suspensions, in which most of the light is transmitted in the spaces between cells, and when dense suspensions are used the results are entirely different. The opacity then appears to depend primarily on the nature of the suspension medium, and scarcely at all on shape.

SUSPENSION MEDIUM	FORM OF CELLS	k
Plasma	Discs	1.00
Plasma + lecithin	Spheres	1.01
Isotonic NaCl	Crenated discs	1.25

The difference is due to the fact that nearly all the light transmitted through a dense suspension is scattered, and that its intensity is dependent on the refractive index difference between the cells and the surrounding medium rather than on the shape of the surfaces. We accordingly have the same opacity for equal numbers in suspensions in plasma and in lecithin-treated plasma, although the shape is different, but a different opacity when the medium is saline, in which the refractive index difference is greater than in the case of the medium being plasma or lecithin-treated plasma.

There is evidence that still other factors contribute to the opacity of these dense suspensions, for in experiments such as the above, in which there is no possibility of variations either in the number or the size of the cells, the same values of k are not always obtained in saline. Since shape has so little effect, and since variations in refractive index difference are unlikely, we have to fall back on as yet unrecognized factors to account for the results.

Discussion. It will be clear from the foregoing that determinations of red cell number or diameter by means of opacimetry are not as reliable as are those made by standard methods. The reason for this is that the opacity of a red cell suspension depends not upon one or even two factors, but upon many, and that, although the various factors (number of cells, area and shape of cells, refractive index differences, etc.) are not of equal importance, any one of them may show a variation which, if not taken into account, may introduce a considerable error into the value found for any other one. A similar difficulty seems to have been encountered in the case of opacimetric measurements of the growth of yeast, where the number of cells cannot be obtained unless changes in their volumes and optical prop-

erties are allowed for (Richards and Jahn, 1933; Stier, Arnold and Stannard, 1934). The consequence is that if we are attempting to find the number of red cells opacimetrically, and assume the cells to have *average* area, etc., and their *normal* shape to be preserved, we may find a discrepancy between the result and that obtained from a properly carried out red cell count to be as great as ±10 per cent; on the other hand, if we wish to improve the precision of the result by allowing for the *actual* cell shape and area, we are involved in such tedious determinations that it is quicker and easier to make a count by a direct method. Similarly, if we are trying to find the red cell area (or diameter), we have first to do an accurate cell count, and then to make certain assumptions about shape, etc., with the knowledge that while these assumptions may apply to the average cell, they do not necessarily apply to the cells of the specimen under examination; a frequent consequence, as has been shown above, is a wrong value for the area or diameter, because the cells of the sample differ in one or many ways from the cells upon which the average values are based.

It is possible, of course, to use the opacimetric method of red cell counting (although not that of finding red cell diameter) for certain purposes, e.g., in investigations where it can fairly be assumed that there are no great variations in cell size, and where an occasional error of 10 per cent is not serious, or in investigations in which only relative numbers are required, but for exact work the method is unreliable. Even for clinical purposes it is unsuitable, for although errors of 10 per cent are by no means uncommon when red cell counts are made as they often are in clinical practice, the possibility of there being considerable variations in cell shape and size in pathological conditions, and therefore even greater errors associated with the method, are too great to be ignored.

SUMMARY

1. The number of red cells in a suspension can be approximately determined by measuring the amount of light transmitted, but the fact that the opacity depends on factors other than cell number, e.g., the area of the cells, their shape, refractive index differences between cells and suspension medium, and other factors which are obscure, makes the method greatly inferior to the more direct methods of red cell counting.

2. While it is true that there is for the average of many individual samples a simple relation between the opacity of suspensions containing equal numbers of red cells and the area or diameter of the individual cells which they contain, the area or diameter of the cells of a given suspension cannot be accurately found from opacity measurements even when the number of cells present is found as exactly as possible by direct counting. Variations in the shape of the cells, etc., peculiar to individual samples, may be such as to lead to an entirely wrong estimate of the individual cell diameter.

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THYROXIN AND TISSUE METABOLISM

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The tendency in this country is to favor the conclusion that tissues from thyroid treated animals have an increased oxygen consumption (Gerard and McIntyre, 1933), in spite of much contradictory evidence particularly among the German workers (Reinwein, 1928; Fleischmann, 1927; Anselmino, 1929; Abelin, 1930).

The work reported in this paper includes and extends material of an earlier publication (Hopping, 1931). It deals with the respiration of the whole blood of the alligator and some of the chemical changes in the blood as they are affected by thyroxin in vivo and in vitro. The expression "thyroxin in vivo" in this paper is understood to mean blood taken from animals previously injected with thyroxin, whereas "thyroxin in vitro" is blood drawn from a control animal to which thyroxin is added after withdrawal.

The blood of the alligator respires at about the same rate as the red cells of the turtle at the same dilution (Ramsey and Warren, 1930) and the cells are less active than those of birds (Barron and Harrop, 1928).

The average oxygen consumption of the blood at 30°C. from control animals throughout the period of observation was 4.6 cu. mm. per gram of fresh whole blood per hour, while the blood from thyroid treated animals respired at the rate of 6.6 cu. mm. per gram of fresh whole blood per hour, an increase of 44 per cent (table 1, series 1 and 2).

The maximal observed increase in the individual tests, however, was over 200 per cent (table 2). This is probably due, in part at least, to the fact that in these animals the thyroid effect is persistent for many months (table 2) so that the effect of repeated injections is cumulative.

Peters and Van Slyke (1932) state that "thyroid extracts have no demonstrable effect on acid base equilibrium." This may be true for warm blooded animals where homeostasis is highly developed. In the alligator, however, we have found a distinct association between thyroid injections and the acid base balance. Thyroid treated animals develop an acidosis shortly after the injections are begun. Later there develops a considerable degree of alkalosis and the buffering power of the blood at this time is much increased above the controls. The regular seasonal variations in blood bicarbonate are seriously disturbed by thyroxin administration.

The study has been complicated by the existence in the alligator of many seasonal variations. The metabolism of the normal whole blood is higher in the late spring and early summer than during the remainder of the year. In warm blooded animals there is recognized to be seasonal variation in the iodine and thyroxin content of the thyroid gland which indicates diminished activity in the summer. In cold blooded animals there is some evidence (Adler, 1920) that there is regression and atrophy of the thyroid gland in the winter although in the summer the gland has a normal appearance. Mann (1916) could not correlate the progress of hibernation with thyroid changes. The increased metabolism of the blood of the alligator at a time, when, according to some workers, the thyroid is more active deserves further study.

Thyroid treated subjects and cases of hyperthyroidism frequently show low respiratory quotients (Abelin, 1930; DuBois, 1927). In this work quotients below 0.70 were met with in the metabolism of the blood following heavy doses of thyroxin (table 2) and also under the action of thyroxin in vitro.

Thyroxin injections increase both the rate of sugar consumption and of lactic acid formation in blood after removal from the body (fig. 4).

The demonstration of the action of thyroxin in vitro is complicated both by the long latent period and by the uncertainty concerning the nature of the physiologically active substance. In these experiments with thyroxin there was no increase in the production of carbon dioxide, in every case this being within the control range. In about half of the cases the oxygen consumption was increased. In the earlier work that was reported (Hopping, 1931) no increases in oxygen consumption were found. This was partly due to the difficulty in establishing control rates because at that time the importance of seasonal variations in metabolism was not recognized.

Methods. All of the experiments were performed on alligators. In all, sixteen animals were used. Eight animals were used for the first 35 experiments. A second set, six females and two males, four of which were used as controls and four for injections, were followed through three years. These animals were between three and four feet long and were of unknown age.

Blood was drawn from the left side of the heart by hypodermic needle into a 20 cc. syringe containing oxalate through experiment 39, and heparin, 1 mgm. per cc. thereafter.

Equal portions of blood (from 5 to 8 cc.) were placed in duplicate tonometers of about 50 cc. capacity, filled with a gas mixture composed of carbon dioxide, oxygen and nitrogen at approximately alveolar tensions. The tonometers were placed for an hour in a water bath at constant temperature and were continually rotated to insure equilibration. One tonometer was then removed and both phases analyzed for carbon dioxide and

oxygen. The gas phase was analyzed in 10 cc. portions with the Haldane apparatus and the blood gases with the Van Slyke manometric method. The second tonometer was kept rotating in the water bath at constant temperature for about twenty-four hours when similar analyses were made. In some cases glucose and lactic acid were also determined.

The change in the composition of the gas phase was from 1 to 4 per cent, the range that is suited for measurement with the Haldane gas analysis apparatus. The changes in the blood were of less importance, since most of the change was in the gas phase, as can be seen from figures 2 and 3. In the making of the blood gas analysis the closed type of pipette (Van Slyke and Neill, 1924) was preferred.

The injections of thyroxin were made by hypodermic needle into the lumen of the left ventricle. Squibb's crystalline thyroxin was used through experiment 72 and Harington's synthetic substance thereafter. No difference in effect was noted.

All of the experiments were run with aseptic technic. Bouillon cultures were made at the close of each experiment. Four of the experiments showed infection and were omitted from the study.

The modern manometric methods for the measurement of tissue metabolism are highly useful where only small portions of tissues are available for study. Where large quantities of a mobile tissue such as blood may be used the method described above was preferable since it gave the opportunity for measuring changes in glucose, lactic acid, pH and bicarbonate, as well as changes in carbon dioxide and oxygen.

Treatment of data. The results have been expressed as simply as possible as cubic millimeters per gram of fresh blood per hour. The red cells compose about 15 per cent of the blood by volume, the red cell count is about 500,000 per cu.mm., with considerable individual variation, the white cells about 8,000 per cu.mm., and the dry weight about one-tenth of the wet weight.

For measuring precision, the mean deviation rather than the standard deviation was utilized for reasons outlined by Scott (1927). For the comparison of observations the mean deviation of the mean was employed.

Results. The results are summarized in table 1. The blood from the thyroid treated animals (series 2, 5 and 6) all show a higher metabolic rate than do the corresponding series of controls (series 1, 3 and 4). Thyroxin in vitro (series 7) brings about a slight increase in oxygen consumption and a marked lowering of the respiratory quotient.

Temperature coefficients of approximately 2.0 were found for both oxygen consumption and carbon dioxide output per 10°C. between 25 and 30. The respiratory quotient of 0.83 was unchanged.

The respiratory metabolism is measurably higher in June and early July than during the remainder of the year (series 3 and 4). No tests were made

in August and September. The thyroid treated animals in June, as during the remainder of the year, gave higher metabolic rates of the blood than the controls during the same period (table 1, series 5 and 6). No correlation between the increased metabolism and the sex of the animal could be made, since the blood from both males and females gave higher rates at this time.

The individual experiments upon the effect of thyroxin in vivo are given in table 2. Several important observations may be made from this table. First, the initial rates are all low, comparable therefore with the controls (series 3, table 1); second, in all cases injections of thyroxin ultimately produced an increased CO_2 output and oxygen intake. Third, in no case was there a return to the initial rate. Further, in experiments 41, 94, 75, 73, 49, 51, 74 and 95, the respiratory quotients were lowered within a few days

TABLE 1

Respiration of the blood of the alligator

SERIES	CHARACTER	NUMBER OF EXPERIMENTS	CU. MM./G. FRESH WEIGHT/HR.		R.Q.
			CO_2	O_2	
1	All controls at 30°C.	22	3.80±6.5%	4.60±6.7%	0.83±0.03
2	All thyroxin in vivo (30°C.)	30	5.05±5.9%	6.63±5.7%	0.76±0.03
3	Controls in winter and early spring	7	3.27±8%	3.77±6%	0.87±0.05
4	Controls in June	8	4.42±6.5%	5.94±6.4%	0.74±0.07
5	Thyroxin in vivo in winter and early spring	23	4.88±7%	6.45±7%	0.76±0.05
6	Thyroxin in vivo in June	7	5.62±9.5%	7.22±6%	0.78±0.07
7	Thyroxin in vitro	11	3.33±8.4%	5.53±15%	0.60±0.09

(from 3 to 8 in all except expt. 95) following the injection. The persistent thyroid effect in experiments 66, 62, and 63a is also worthy of attention.

The control animals were used as often as the thyroid treated animals so that the increased metabolism can hardly be attributed to hemorrhage. In the few experiments that have been directed to the problem of the effect of hemorrhage little effect upon oxygen consumption was shown although the red cell count was decreased and the white cell count increased (table 3).

Immediately following the injections of thyroxin the animals lost weight. During the intervals between injections, when these were of several months' duration, the injected animals increased in weight, 44 per cent, as compared with only a 3 per cent increase in the controls. At the end of the three year period the thyroid treated animals were 30 per cent heavier than at the beginning whereas the controls had lost 12 per cent. The red

TABLE 2

Individual experiments showing the effect of thyroxin in vivo on the metabolism of the blood

NUMBER OF EXPERIMENT	DATE	QUANTITY OF THYROXIN INJECTED AND THE DATE	CU. MM./G. FRESH WEIGHT/HR.		R.Q.
			CO_2	O_2	
		Animal 1			
36	11/20/30	Initial value	3.08	3.42	0.90
41	12/18/30	3 mgm. 12/15/30	4.65	6.67	0.70
44	1/15/31	3 mgm. 1/5/31 4.5 mgm. 1/9/31	10.03	12.40	0.83
		per cent increase over initial value	252	262	
66	11/19/31	10 mos. after last injection	6.15	6.71	0.91
72	3/31/32	1 mgm. daily doses to 5 mgm.	3.83	4.80	0.80
94	11/29/32	15 mgm. 11/21/32 H	6.26	9.21	0.68
		Animal 2			
40	12/15/30	Initial value	2.82	3.39	0.83
48	3/19/31	3 mgm. 3/16/31	4.21	4.10	1.03
57	6/15/31		7.11	7.57	0.94
63a	11/ 9/31	8 mos. after last injection	4.85	5.71	0.85
75	4/ 7/32	6 mgm. 4/4/32 H	6.26	10.54	0.60
		per cent increase over initial value	122	211	
84	6/13/32		7.86	7.84	1.02
		Animal 3			
43	1/21/31	4.5 mgm. 1/9/31	8.58	9.98	0.86
69	1/28/32	5 mgm. in 1 mgm. daily doses	4.48	4.67	0.96
73	3/24/32	10 mgm. 3/21/32 H	4.88	7.30	0.67
97	12/19/32	10 mgm. 12/14/32 H	4.97	6.21	0.80
		Animal 4			
39	12/ 9/30	Initial value	2.66	2.69	0.99
49	3/23/31	3 mgm. 3/16/31	2.52	3.23	0.78
51	4/27/31	10 mgm. 4/21/31	5.46	8.55	0.64
		per cent increase over initial value	105	218	
62	10/29/31	6 mos. after last injection	6.63	6.98	0.95
71	2/ 8/32	5 mgm. in 1 mgm. daily doses	4.66	4.57	1.10
74	4/ 6/32	5 mgm. 3/24/32 H 2.5 mgm. 4/1/32 H	2.71	6.60	0.41
87	6/23/32		4.34	5.53	0.81
91	10/27/32	10 mgm. 10/17/32 H	4.27	4.45	0.96
95	12/ 5/32		5.53	7.78	0.71

H = Harington's thyroxin, all others Squibb's crystalline thyroxin.

cell count in the thyroid treated animals increased over 40 per cent. These animals had better appetites, were more active and more dangerous to handle than the controls.

The data allowed the calculation by the Henderson-Hasselbalch equation of the pH and of the bicarbonate of the blood, and thus also of the changes in these properties during the metabolic period. The absorption coefficient of 0.511 for CO_2 in whole blood was used. The value for pK_1 in the Hasselbalch equation was taken as 6.14 at 30°C. from the work of Austin, Sundermann and Camack (1927).

The data from the three years' study of the controls and of the thyroid treated animals are given in figures 1, 2, and 3. The oxygen tensions were about 130 mm. and warrant the conclusion that these changes in bicarbonate were not due primarily to changes in the percentage saturation of the hemoglobin.

During the first half of the year of 1931 (fig. 1) an acidosis is apparent in the thyroid treated animals. This gradually disappeared so that during

TABLE 3

Hemorrhage

NUMBER OF EXPERIMENT	NUMBER OF THE ANIMAL	DATE	CU. MM./G. FRESH WEIGHT/HR.		R.Q.	RED CELL PER CU. MM.	WHITE CELL PER CU. MM.
			CO_2	O_2			
63a	2	11/ 9/31	4.85	5.71	0.85	568,000	11,000
65	2	11/16/31	5.79	6.98	0.83	553,000	14,000
67	2	11/23/31	4.74	4.65	1.02	454,000	19,000

Total of 42 cc. drawn in two weeks.

the second year the level of the bicarbonate in the thyroid treated animals became progressively higher than in the controls, reaching a maximum in the winter when the controls were at their lowest level.

Other data show that at the beginning of the treatment the buffering power of the oxygenated blood of the controls and of the thyroid treated animals was the same. During the period of compensation, the buffering power of the blood of the thyroid treated animals became progressively higher than that of the controls. Thus, in the final period, blood from control animals held 43 vols. per cent of bicarbonate at a pH of 7.6, whereas 59 vols. per cent were held by blood from the thyroid treated animals at the same pH.

In figure 2 the closed circles represent the group averages of the initial values of the control blood at various seasons; the open circles, the values after the blood had been incubated for 24 hours. The dotted lines show the shift during the period of incubation. In the late winter and early spring there is a higher pH and CO_2 content of the blood than during the

fall and early winter months. This cyclical change was repeated regularly from year to year without exception, the data for the third year falling between those of the first and second years. Thus there was no tendency in the controls during the period studied toward the disruption of this cycle of an alkalosis in the spring and an acidosis in the early winter.

Comparison of this figure with figure 3 which represents similar data for the blood with thyroxin in vivo, brings out several differences. The entire mass of data in both figures 2 and 3 falls within the pH range recognized as compatible with life as met with in mammalian forms. Thyroxin in vivo, however, during the incubation period induces a markedly greater change in both pH and CO_2 tension. In addition thyroxin in vivo apparently destroys the seasonal change in bicarbonate and pH characteristic

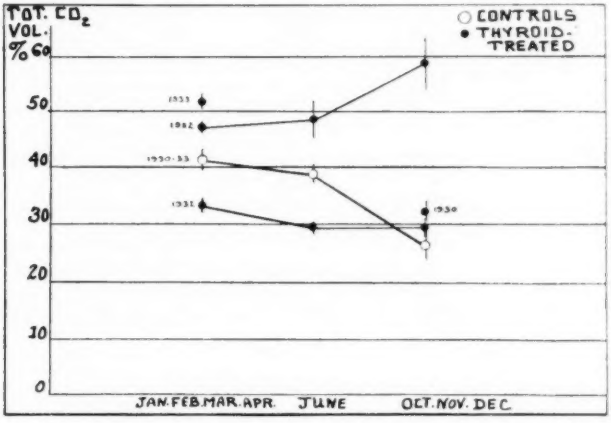

Fig. 1. Seasonal changes in total CO_2 in blood from controls and from thyroid treated animals. CO_2 tension 30 mm. approximate. O_2 tension 130 mm. approximate.

of the controls. With the injections there develops an early acidosis (*2* and *3* in fig. 3) which is followed by a marked alkalosis (*4*, *5*, *6* and *7* in fig. 3).

The carbon dioxide that is produced during the period of incubation is not absorbed by the blood according to the CO_2 absorption curve of oxygenated whole blood (dotted line in fig. 2). The smaller amount of CO_2 held by the blood at the end of the incubation period indicates the formation of some acid which then combines with the reserve alkali displacing CO_2. A few determinations indicate that lactic acid is formed during the incubation period in greater degree by the blood from thyroid treated animals than by the blood from the controls. The initial concentration of lactic acid was in some cases lower in the blood from thyroid treated animals than in the blood from the controls. During the metabolic period,

however, the control blood formed 1.9 milliequivalents of lactic acid per liter per 24 hours, whereas more than twice that amount, 4.2 milliequivalents, is formed in the same time under the action of thyroxin in vivo.

Figure 4 is a graphic representation of glycolysis, lactic acid and CO_2 formation in control blood, in blood with thyroxin in vivo, and in blood with thyroxin in vitro. The quantities represented are milligrams per hour per liter of blood. The heavy lines represent glucose consumed (glycolysis), the diagonal hatching, lactic acid, the dotted portions, the amounts of CO_2 produced calculated as glucose, the open space representing undetermined intermediate products. The dotted lines through the controls and

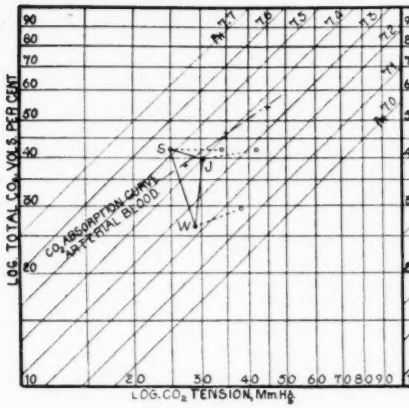

Fig. 2

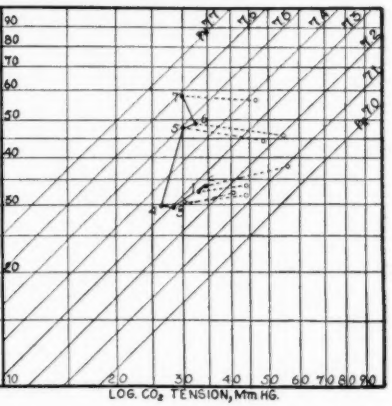

Fig. 3

Fig. 2. Seasonal variation in total CO_2. Controls. Arterial blood at 30 mm. (approximate). *S*, January, February, March, April; *J*, June; *W*, October, November, December (from June 1930 March to 1933). Dotted lines represent changes during the metabolic periods.

Fig. 3. Thyroid treated animals. As in figure 2. *1*, winter 1930; *2*, *3* and *4*, 1931; *5*, *6*, and *7*, 1932. Initial and final values of metabolic periods are connected by dotted lines.

the thyroid treated blood indicate means for glycolysis obtained in other experiments. Since the respiratory quotients in the experiments with thyroxin in vitro were below 0.70 it is assumed that no glucose was burned to carbon dioxide and water.

This group of experiments indicates that thyroxin in vivo affects carbohydrate metabolism by increasing the rate of conversion of glucose to lactic acid, and by accelerating the disappearance of other intermediate products of carbohydrate metabolism. Thyroxin in vitro increases glycolysis considerably but lactic acid formation only slightly.

The fundamental problem of the action of thyroxin in vitro has been

studied by many different workers, by many different methods and with conflicting results (excellent reviews of the work in this field have been

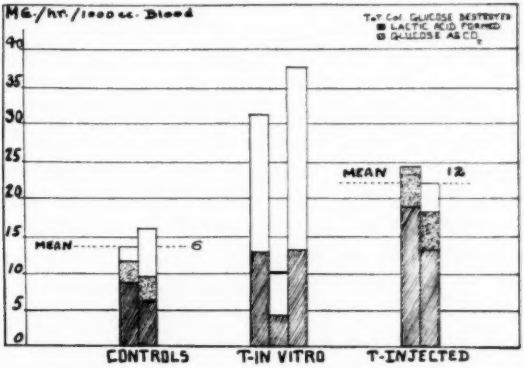

Fig. 4. Glucose consumption and lactic acid formation in blood from controls and from thyroid treated animals.

made by Reinwein et al., 1928, Robles, 1931, and Gerard and McIntyre, 1933).

The results of our experiments with thyroxin in vitro are summarized in table 4. In these experiments there was never an increase in CO_2 production. In seven of the eleven experiments there was an increase in oxygen consumption beyond the deviations of the controls.

TABLE 4

Thyroxin in vitro

SERIES	NUMBER OF EXPERIMENT	CONCENTRATION OF THYROXIN G./CC. OF BLOOD	CU. MM./G. FRESH WEIGHT/HR.		R.Q.
			CO_2	O_2	
Controls in June	8		4.42±6.5%	5.94±6.4%	0.74±0.07
Additions in June	2	3×10^{-7}	4.43±9.0%	10.0 ±17%	0.44±0.05
Controls at 30°C.	7		3.27±8.0%	3.77±5.5%	0.87±0.05
Additions without increase	4	3×10^{-6} to 7×10^{-7}	3.52±8%	3.4 ±7%	1.03±0.07
Additions with increase	5	7×10^{-3} to 6×10^{-6}	2.75±15%	5.46±7%	0.50±0.07

The latent period in the action of thyroxin according to Kunde (1927) is from 7 to 12 hours following intravenous injections in the dog. The incubation period in these tests was about 24 hours so that sufficient time was allowed for the action to develop although maximal effects could not be expected. In one case where there was a preliminary incubation period of

24 hours before the metabolism test was begun, the oxygen consumption was much greater.

It is noteworthy that the low respiratory quotients reported as frequently occurring in hyperthyroidism (DuBois, 1927; Abelin, 1930) have been found in these experiments with thyroxin in vivo, and that they are still more apparent under the action of thyroxin in vitro. It is frequently assumed that low quotients are due only to faulty technic. There was, however, in this work, no more technical reason for doubting the low quotients found in the thyroid blood than to doubt the normal quotients found in the controls. Thus, the average for all of the controls (table 1, series 1) is 0.83 ± 0.03; that for thyroxin in vivo, 0.76 ± 0.03 (table 1, series 2) and for thyroxin in vitro 0.60 ± 0.09 (table 1, series 7).

Histological preparations of the thyroid glands of the controls and of the thyroid injected animals were made at the termination of the study in January, 1933, when the animals were killed. All of the glands in both control and injected animals were in the typical resting condition.

SUMMARY

We have made the following observations:

Thyroxin in vivo accelerates the metabolism of the blood of the alligator. Carbohydrate metabolism is accelerated through an increased consumption of glucose and by an increased formation of lactic acid. Intermediate substances between glucose and CO_2 other than lactic acid are decreased. Thyroxin in vivo and in vitro lowers the respiratory quotient.

There is a seasonal increase in metabolism in early summer.

Thyroxin in vivo affects the acid base balance by producing an initial acidosis followed by a progressive alkalosis, due probably to the increased production of CO_2 and to the development of an increased buffering power of the blood.

Thyroxin is only slowly destroyed in these cold blooded animals, an increased metabolism being observable six months and more after injection.

Thyroxin in vitro increases the oxygen consumption in some cases but does not increase CO_2 production.

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THE EFFECT OF FEEDING HIGH AMOUNTS OF SOLUBLE IRON AND ALUMINUM SALTS[1]

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Studies which have been in progress in this laboratory for several years dealing with the availability of various forms of calcium for bone formation included a number of determinations on calcium aluminum silicates and calcium iron silicates. These salts were included in an attempt to find forms of calcium which were unavailable to the body. However, when they were fed at fairly high levels to chicks on rations containing adequate vitamin D, a more severe rickets than is usually obtained on a low calcium intake was observed. We immediately suspected that the deficiency was dependent upon a decreased phosphorus utilization due to the large amounts of aluminum and iron ingested.

Some work has been done on the effect of iron and aluminum on phosphorus assimilation. Cox, Dodds, Wigman and Murphy (1) using guinea pigs and rabbits found that rations containing soluble aluminum salts in excess of the total phosphorus in the diet produced a severe lowering of the blood phosphorus and bone ash. Ferric salts produced similar but less marked changes. They found that the addition of sodium acid phosphate would prevent these changes and concluded that the effects were due to a precipitation of ferric and aluminum phosphates in the tract. Brock and Diamond (2) working with rats found that the addition of large amounts of ferric or ferrous salts to a non-rachitogenic diet produced rickets. They also showed that additions of inorganic phosphate would prevent the rickets. Guyatt, Kay and Branion (3) obtained somewhat similar results with beryllium.

In this paper we wish to present the results obtained from iron and aluminum feeding in the case of chicks.

EXPERIMENTAL. Day-old White Leghorn chicks were used in all the work. They were housed in the usual way in warmed cages with wire screen bottoms. The basal ration used was the rachitic ration described by Hart, Kline and Keenan (4) supplemented with 1 per cent cod liver oil. This ration contains 0.91 per cent Ca, 0.51 per cent P and when supplied

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with adequate vitamin D allows rapid growth and normal bone formation. Detailed studies on blood calcium and phosphorus and bone ash in chicks on various modifications of this ration have been published by Elvehjem and Kline (5).

For bone analyses the left tibia was removed from each chick and stored in alcohol until a group of bones had collected. The bones were then crushed, extracted with hot alcohol for 72 hours, dried, and ashed in an electric furnace. The percentage of ash was calculated on the moisture- and fat-free basis. The blood calcium was determined on the serum by Clark and Collip's modification of the Kramer and Tisdall method and inorganic phosphorus by the Fiske and Subbarow method.

When chicks were placed on the basal ration in which the $CaCO_3$ was replaced by an equivalent amount of calcium as fused calcium silicate ($CaOAl_2O_3(SiO_2)_2$) the chicks grew fairly well but the bone ash at 5 weeks

TABLE 1

The effect of iron and aluminum salts on bone ash and blood phosphorus

GROUP NO.	SALTS IN RATION					BONE ASH	¡P PER 100 CC. SERUM	Ca PER 100 CC. SERUM
	$Fe_2(SO_4)_3$	Fe	$Al_2(SO_4)_3 \cdot 18H_2O$	Al	Na_2HPO_4			
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*	*mgm.*
1	3.22	0.90				23.41	4.6	12.2
2	2.42	0.67				25.47	4.4	12.2
3	1.61	0.45				27.68	2.7	12.7
4	3.22	0.90			5.77	44.51	6.8	10.4
5			5.37	0.44		31.74	3.5	10.6
6			4.03	0.33		24.61	2.4	12.3
7			2.69	0.22		25.98	2.3	14.0
8			5.37	0.44	5.77	44.72	6.2	10.5

of age was only 37 per cent. When the amount of silicate was doubled in an attempt to supply more available calcium the bone ash was only about 27 per cent at 5 weeks. The addition of the larger amount of the silicate had a very deleterious effect on bone formation. Additional results obtained with silicate are given in another paper (6).

In the next trial the calcium was supplied as calcium carbonate but sufficient ferric citrate or aluminum sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$) was added to unite with the total phosphorus contained in the ration as $FePO_4$ or $AlPO_4$. In spite of the fact that the chicks received adequate calcium and vitamin D those fed the soluble aluminum salt exhibited severe symptoms of rickets as early as the tenth day and were all dead by the end of the third week. The bones contained an average of 31.74 per cent ash. The group on ferric citrate survived five weeks but had a bone ash of only 34.12 per cent. Both the salts prevented normal bone formation although the

iron in the form of ferric citrate did not produce as severe a condition as the more soluble aluminum salt.

Since ferric citrate is somewhat insoluble and the iron less available to unite with the phosphates of the ration another experiment was conducted using ferric sulfate and aluminum sulfate. In this series three different levels of the salts were used. The highest level in both cases corresponded to equal amounts of iron or aluminum and phosphorus calculated on the basis of $FePO_4$ and $AlPO_4$. The intermediate level supplied 0.75 and the lowest level 0.5 of this amount. In addition one group on high aluminum and one on high iron received sufficient disodium acid phosphate to react with the added metals. The amount of each salt added is given in table 1. All groups received 1 per cent cod liver oil. Ten chicks were used in each

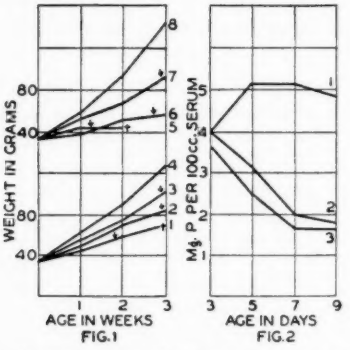

Fig. 1. Average growth records for chicks on iron and aluminum salts. 1, high iron; 2, intermediate iron; 3, low iron; 4, high iron plus phosphate; 5, high aluminum; 6, intermediate aluminum; 7, low aluminum; 8, high aluminum plus phosphate. Arrow indicates time at which majority of chicks exhibited severe rickets.

Fig. 2. Daily changes in blood phosphorus. 1, basal ration; 2, basal + 4.03 per cent $A_2(SO_4)_3 \cdot 18H_2O$; 3, basal + 2.42 per cent $Fe_2(SO_4)_3$.

group and the averages of the growth records for each group are given in figure 1. The arrow indicates the time at which the majority of the chicks exhibited rickets. It is readily seen that all chicks getting the iron or aluminum salt alone developed rickets between 1 and 3 weeks of age. The rickets developed in all cases at an earlier time than in chicks reared on a diet deficient in vitamin D alone. Rickets is very seldom observed in chicks on a standard rachitic ration before the fourth week.

Most of the chicks on the high aluminum were dead at 2 weeks of age while those on the high iron survived about 3 weeks. The chicks on the lower levels did not succumb but all showed signs of rickets. All remaining chicks were killed at 3 weeks of age for bone and blood analysis. The results are given in table 1. All levels of iron and aluminum produced a

very low ash content of the bones. The ash in the bones from the chicks on the highest level of aluminum was higher than the other groups, probably because these animals died very early. The values for the other groups were lower than those usually obtained for chicks of the same age deprived of vitamin D. The chicks receiving added phosphate gave bones with normal ash content. The inorganic phosphorus content of the blood of these chicks was also high while the animals receiving the iron or aluminum alone showed very low values. The group receiving the lowest level of iron and aluminum gave values slightly above 2 mgm. per 100 cc. of serum. The figures for the high levels ranged from 3.5 to 4.6 mgm. due undoubtedly to a dehydration of the blood during the period of very severe rickets. There was no drop in blood calcium. The values for calcium were increased somewhat when the blood phosphorus values were low.

TABLE 2

The effect of feeding iron and aluminum salts for 11 days on bone ash and blood phosphorus

GROUP NO.	SALTS IN RATION				BONE ASH	P PER 100 CC. SERUM
	$Fe_2(SO_4)_3$	Fe	$Al_2(SO_4)_3 \cdot 18H_2O$	Al		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*
1	3.22	0.90			25.38	1.7
2	1.61	0.45			29.54	2.2
3			5.37	0.44	25.93	3.5
4			2.69	0.22	28.34	2.1
5					38.33	5.7

In order to study the possible effect of dehydration on the blood results and the rate at which the iron and aluminum affect the blood phosphorus another series of chicks was run for a shorter period of time. In this series only the highest and lowest levels of iron and aluminum were used and the animals were killed at 11 days of age. The results are given in table 2. Even at this early age the phosphorus values are very low as compared with the normal. Again the highest blood phosphorus values were found in the high aluminum group. Only two chicks in this group were able to stand when the blood sample was taken. They had consumed very little food the last two days. There was a spread of at least 10 per cent in the bone ash between the normal chicks and those fed iron and aluminum.

Three additional groups of chicks were started on the normal ration and on the intermediate levels of iron and aluminum. Five chicks from each group were killed for blood analyses at 3, 5, 7, and 9 days of age. The results are given in figure 2. The blood phosphorus at 3 days of age is 3.8 to 4.0 mgm. per 100 cc. serum in all groups. By the fifth day there is a

definite drop in the iron and aluminum groups and an increase in the normal group. Values as low as 1.5 to 1.7 are obtained by the ninth day in the groups without added phosphate.

DISCUSSION. The results presented in this paper demonstrate conclusively that the addition of soluble iron and aluminum salts to a natural diet produces very severe rickets in chicks. Levels of iron and aluminum sufficient to unite with one-half the total phosphorus in the ration as $FePO_4$ or $AlPO_4$ will reduce the bone ash to 25 to 27 per cent at 3 weeks of age. Higher levels produce rickets in a shorter time. The rickets is undoubtedly due to a lowered blood phosphorus which may be observed within 5 days after the iron or aluminum is added and which may fall to levels of 1.5 mgm. per 100 cc. of serum.

From a practical point of view these observations may not be of great significance because iron and aluminum ingestion will never reach the levels used in the work in ordinary diets. However many workers are now recommending the use of very high doses of iron in the treatment of secondary anemia, dosages equivalent to the highest level used in these experiments. We have called attention to this fact in a recent paper (7) on the use of iron in the treatment of anemia in children. Precautions against high ingestion of iron should be made not only in the case of infants but also with adults because any change in the diet which will reduce the blood phosphorus to levels reported here surely will affect other biological processes in addition to bone formation.

The fact that one-half of the amount of iron and aluminum needed to combine with the total phosphorus in the diet will produce severe disturbances indicates that only a portion of the phosphorus in the ration is available. The possibility of variations in the availability of phosphorus in different rations was suggested in an earlier paper (4). Recently Bruce and Callow (8) point out that certain forms of phosphorus are less available than others. The use of diets high in iron or aluminum may be valuable for the determination of the availability of phosphorus compounds.

SUMMARY

1. Day-old chicks placed on a normal ration to which was added large amounts of soluble iron or aluminum salts (0.9 per cent Fe, 0.44 per cent Al) developed severe rickets in one to two weeks and were all dead within 3 weeks after being placed on the ration.

2. Levels of iron and aluminum salts equivalent to 0.5 and 0.75 of the amount necessary to unite with the total phosphorus in the ration as $FePO_4$ and $AlPO_4$ reduced the bone ash to about 25 per cent and the blood phosphorus to 2 to 4 mgm. per 100 cc. of serum at three weeks of age. Similar results were obtained after the chicks had been on the rations only 11 days.

The addition of sodium acid phosphate in amounts sufficient to unite with the added metals allowed rapid growth and normal bone formation.

3. A definite drop in blood phosphorus was observed as early as the fifth day and values as low as 1.5 to 1.7 mgm. per 100 cc. serum were obtained by the ninth day after the addition of iron or aluminum salts.

4. The possible danger resulting from the use of high doses of iron in the treatment of hypochromic anemias is discussed.

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RHYTHMIC CHANGES IN DUODENAL MOTILITY ASSOCIATED WITH GASTRIC PERISTALSIS

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Whether gastric peristalsis significantly influences duodenal motility has not been definitely determined. It is generally admitted that some influence passes over the pylorus from the stomach, but its effect on the duodenum has been variously described and interpreted.

Although these variations in interpretation are indicated in the reviews by Ivy (1927) and Alvarez (1929), the more pertinent observations are briefly the following. Joseph and Meltzer (1910) observed that duodenal rhythmic activity and tonus are temporarily inhibited during each contraction of the pyloric antrum and suggested that this phenomenon is identical in design with the receptive relaxation of the stomach described by Cannon and Lieb (1910). Later Wheelon and Thomas (1922) obtained graphic results which apparently justified the conclusion that the gastric wave passes over the pylorus to the duodenum and there initiates duodenal peristalsis. Their observations were made on anesthetized animals; consequently their results were somewhat irregular and their conclusions probably subject to a corresponding degree of uncertainty. The same criticism may be applied to the observations of Alvarez and Mahoney (1923), who noted that an increase in the tonus of the duodenum often occurred about the time the gastric wave reached the pylorus. Uncertainty concerning the initial inhibition of the duodenum described by Joseph and Meltzer is also implied in the opinion expressed by Ivy and Vloedman (1925); they wrote that in a majority of instances the distal portion of the duodenal cap contracts after a brief pause following the arrival of a vigorous gastric wave at the pylorus. In view of the existing uncertainty it appeared worth while to reinvestigate the problem by more appropriate methods.

METHOD. Dogs were prepared with cannulated gastric and duodenal fistulas as described recently (Thomas, Crider and Mogan, 1934). Apparatus and other details of the method were the same as in the previous study except that a duodenal balloon was added, arranged in the same manner as the gastric balloon. The gastric and duodenal balloons were placed as near as possible to the pylorus.

Simultaneous graphic records of gastric peristalsis, pyloric activity, and duodenal motility were obtained while the stomach was empty, during feeding and for several hours thereafter. The results to be described were obtained in fourteen experiments on two dogs. They are not complicated by the effects of anesthetics or other drugs.

RESULTS. *During digestion* practically every gastric peristaltic wave recorded appeared to exert a profound influence on the motility of the first part of the duodenum, affecting principally the amplitude of the rhythmic contractions. While the antral peristaltic wave was approaching the pylorus the duodenum was either inactive or performing only feeble rhythmic contractions. Immediately after the antral wave reached the pylorus one, two or three duodenal contractions occurred. The first or, less frequently, the second contraction to follow the antral wave was nearly always the highest by a considerable margin. Succeeding contractions diminished progressively in amplitude in the order of their occurrence. Ordinarily, in the dog, there is time for four rhythmic contractions of the duodenum during each antral cycle but the fourth contraction was commonly suppressed and its place taken by relaxation. Often the third contraction was also absent, and sometimes the second as well so that a single contraction of the duodenum followed the gastric wave. Typical records are shown in figure 1.

Tone changes in our experiments were moderate but were in the same direction as the coincident changes in amplitude of contraction.

The behavior described is most evident in the first few centimeters of the gut. Records obtained from balloons in the duodenum at different distances from the pylorus show that there is a progressive decrease in the gastric influence as the distance from the pylorus increases. There was, in our experiments, no evidence of a gastric rhythm in the lower part of the duodenum.

It may be inferred that the gastric influence on the duodenum is predominantly inhibitory from the fact that, in general, the activity of the duodenum near the pylorus where the gastric influence is most marked is less than that lower down where it is diminished or absent.

Records obtained simultaneously from two balloons 10 cm. apart show that the change associated with gastric peristalsis travels over the duodenum at the rate of approximately 10 cm. per second. The rates observed averaged 11.26 cm. per second in one animal and 9.18 cm. in the other. This is the same as the rate of conduction of descending inhibition in the intestine as determined by Bayliss and Starling (1899).

Figure 2 shows a result typical of experiments designed to determine whether the duodenal activity that is associated with gastric peristalsis could be identified with intestinal peristalsis. The activity shown in the bottom curve conforms to the description of peristalsis by Bayliss and

Starling. This record shows little resemblance to the record above it showing responses of the duodenum to the gastric cycle. However, the differences are mainly quantitative; in the record of peristalsis the contraction phase lasts longer than the preceding inhibition whereas the reverse is true of the record above. The greater total duration of the activity over the lower balloon is probably due to a slower rate of travel. From the data available it appears that the activity in the first part of the duodenum

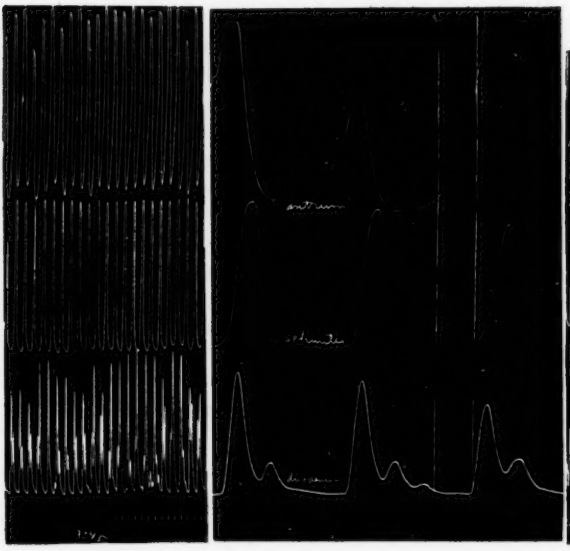

Fig. 1

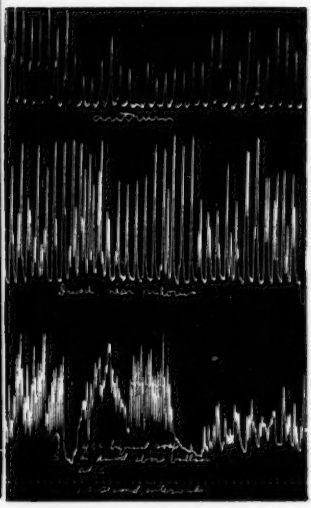

Fig. 2

Fig. 1. Slow and fast drum records of antral (top), pyloric (middle), and duodenal (bottom) activity during digestion. The antral and duodenal balloons were near the pylorus. Time is marked in 10 second intervals. Vertical lines mark simultaneous points.

Fig. 2. Record to show the difference between the activity associated with gastric peristalsis, in the first part of the duodenum (middle record) and the activity, believed to be peristalsis, induced by injecting soap solution into a lower portion of the duodenum (bottom record).

travels about 300 times as rapidly as ordinary intestinal peristalsis. The two types of motility resemble one another in that each comprises a phase of inhibition followed by a phase of contraction, traveling aborally.

In agreement with Alvarez and Mahoney we found that in a large majority of instances the duodenal contraction of greatest amplitude began at the same time as the contraction of the pyloric sphincter and not after a delay as Wheelon and Thomas indicated. Occasionally the duodenal contraction appeared to begin slightly in advance of the pyloric contraction.

When the stomach was empty a greater variety of duodenal activity was evident. Often there was marked rhythmic activity of the first part of the duodenum with only haphazard changes in tonus and amplitude not suggestive of a gastric rhythm. Generally under such conditions there was little or no activity of the stomach but if regular peristalsis appeared, as it occasionally does even in the "empty" stomach under our experimental conditions, the duodenal activity showed unmistakable evidence of the gastric influence, similar to that observed during digestion.

We were able to confirm the observation of Ivy and Vloedman (1925) that the duodenum participates in hunger activity. Our records obtained during hunger activity are practically identical with their published records.

While the animal was taking food there was nearly always complete cessation of activity in the first part of the duodenum. In a typical case, when hunger contractions were present before feeding the marked activity of the duodenum characteristic of hunger was suddenly interrupted with the first presentation of food. The stomach and frequently the pylorus were inhibited also. The duration of the inhibition in the duodenum was variable and the recovery frequently gradual so that in many instances half an hour or more elapsed before the strongest duodenal contractions regained the amplitude which characterized the hunger activity. The first activity to appear in the duodenum after feeding was associated with the gastric cycle and commonly consisted of a single contraction following the arrival of each gastric wave at the pylorus.

The duodenal inhibition during feeding is evidently associated with the receptive relaxation of the stomach and is not to be confused with the receptive relaxation of the duodenum suggested by Joseph and Meltzer which is a rhythmical phenomenon associated with gastric peristalsis.

Discussion. The phenomena described may be given at least two quite different theoretical interpretations. The rhythmic changes in the duodenum associated with gastric peristalsis may, we think, be fully explained as comprising merely quantitative modifications of otherwise independent duodenal activity. The results indicate that the gastric peristaltic wave is preceded by a wave of inhibition which, whether or not it affects the gastric muscle, profoundly influences the activity of the first 15 or 20 cm. of the duodenum. The contractions that regularly follow the arrival of the gastric wave at the pylorus may, we think, be satisfactorily interpreted as independent activity of the duodenal muscle which is momentarily freed from inhibition. The new facts elicited in this investigation appear to us to be favorable to this interpretation.

On the other hand, there is nothing to exclude the possibility that the inhibitory influence is followed by an influence of opposite sign, i.e., a wave of excitation transmitted from the stomach. If there is such a wave

the result cannot readily be differentiated from peristalsis and it is proper to speak of the transmission of the gastric peristaltic wave over the pylorus.

Other interpretations have been suggested but most of them can be excluded by our results. For example, we found no support for the suggestion by Alvarez and Mahoney (1923) that the intestine has a normal rhythm, approximating in frequency the gastric rhythm, which can occasionally be "captured" by the stomach. The relation between gastric and duodenal rhythm is not occasional and approximate but constant and exact. Ivy's (1927) suggestion that the duodenal rhythm may be due to rhythmic stimulation by the gastric discharge does not explain the appearance of a gastric rhythm in the duodenum when the stomach is empty.

Of more practical interest are the obvious facts that a period of relative inactivity in the first part of the duodenum occurs regularly in such relation to gastric peristalsis as to serve the purpose of accommodating the duodenum to the reception of the gastric discharge (a receptive relaxation of the duodenum), and that this is followed by recovery or an increase in activity which travels from the pylorus downward and so must assist in the forward progress of the duodenal contents.

SUMMARY. Simultaneous graphic records of gastric peristalsis, pyloric activity and duodenal motility show that:

1. During digestion as the gastric peristaltic wave approaches the pylorus there is, regularly, marked inhibition of the rhythmic contractions of the first 15 or 20 cm. of the duodenum. Recovery of activity in the duodenum begins at the same time as the contraction of the pyloric sphincter that follows each gastric wave.

2. The changes described start at the pylorus and travel downward at a speed characteristic of the spread of descending inhibition (10 cm. per sec.) but more rapidly than ordinary peristalsis.

3. Records of the activity of the duodenum between antral cycles do not resemble records of intestinal peristalsis.

4. The gastric influence appears, on the whole, to diminish the activity of the part of the duodenum affected as compared to the activity of other unaffected parts.

5. The duodenum is inhibited along with the stomach while food is being taken.

CONCLUSION

We agree with Joseph and Meltzer that there is a receptive relaxation of the duodenum associated with each gastric peristaltic wave. We are unable to determine whether the subsequent contraction is in part due to the passage of the gastric wave over the pylorus or entirely to recovery of the duodenum from inhibition.

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LIVER AMYLASE: THE EFFECT OF NUTRITION AND OF HORMONES[1]

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In a previous communication (1) are reported the effects of several hormones upon the amylase activity of tumors borne by mice. Insulin and thyroxin were found to increase the apparent concentration (expressed as a so-called α-value (1)) of amylase, whereas epinephrine showed no effect. This paper gives the results of a similar assay of liver in animals which received injections of thyroxin, insulin, and epinephrine respectively.

EXPERIMENTAL PLAN. At the outset of this work, it was seen that there was a complicating factor in liver not found in tumor. Whereas the activity-value for tumor was not affected by the state of nutrition of the animal, the α-value for liver, on the other hand, exhibited wide variation in animals whose state of nutrition had not been rigidly controlled. Consequently, controlled feeding experiments were performed. Following a 48-hour period of fasting, the mice were weighed and fed a measured amount of 30 per cent glucose solution by stomach tube. This method of feeding has been described by Salter, Robb, and Scharles (2). The dose of glucose used in all these experiments was 0.75 gram per 100 grams of mouse. Following feeding, one-half of each group of animals served as controls and the other half received insulin or adrenalin. The animals were killed at varying periods after feeding.

In the experiments on thyroxin, the animals had been previously rendered thyrotoxic. They, together with their controls, were fasted only 24 hours prior to the determinations, since this was the maximum time which they could consistently survive starvation. They were then fed in the manner just described, and the apparent amylase content of their livers determined subsequently.

The animals used in this work were healthy black mice of a pure strain, inbred for over ten years. The mice were from three to five months old, and weighed 18 to 22 grams after fasting. In any single experiment the controls and injected mice were always of approximately the same age and weight and had been kept under identical conditions.

[1] This work was made possible by grants from the International Cancer Research Foundation and the Ella Sachs Plotz Foundation.

ANALYTICAL METHOD. The animals were killed by guillotining with large shears. In a few cases, simultaneous amylase and glycogen determinations were made on the same liver. The liver was quickly removed, and approximately one-half was dropped into a tared centrifuge tube containing 2 cc. of 40 per cent potassium hydroxide. Glycogen was determined by the method described by Good, Kramer and Somogyi (3). The other half was weighed to the nearest decigram, and an extract prepared for determination of the α-value. When amylase alone was to be determined, the liver was removed as rapidly as possible, the gall bladder was evulsed with forceps, and the liver was weighed to the nearest tenth of a gram.

The tissue was then ground in a mortar with the addition of sand until a homogeneous paste was obtained. Distilled water was added (1 cc. of water per gram of liver) and the grinding continued for two to five minutes. To this paste was added 0.9 per cent saline (eight volumes of saline per gram of liver). The grinding was continued for a minute or two and then the entire mass was transferred to a centrifuge tube and centrifuged for 15 minutes at 2000 r.p.m. Glycogen determinations were made on the solutions before and after incubation with the liver extract by Pflüger's method as modified by Slosse (4), and were fully described in our previous paper on tumor amylase (1). Sugar determinations on the acid-hydrolysate were done by a modified Shaffer-Hartmann method, using 1 gram of potassium iodide per liter of reagent (5). Rabbit liver glycogen, prepared by the method of Alsberg and Sahyun (6), was used as substrate.

The activity of liver extract was measured as follows: One cubic centimeter of a 2 per cent glycogen solution in 0.02 Molar phosphate buffer at pH 6.2 was measured into each centrifuge tube. This was followed by 0.2 cc. of the tissue extract and 0.8 cc. of physiological saline. Thus there was obtained a final mixture of 1 per cent liver extract in 1 per cent glycogen and 0.01 Molar phosphate. Each tube was stoppered and placed in a water bath at 45°C. for exactly 3 hours. After incubation, 2 cc. of 40 per cent potassium hydroxide were added and the glycogen determinations made in the same tube. A blank determination was made on a similar mixture immediately after the addition of the extract to the glycogen. Determinations of amylolytic activity for samples of the same liver extract seldom varied more than the deviation to be expected in the analysis for glycogen. Results which showed a loss of less than 200 mgm. per cent or more than 800 mgm. per cent glycogen after incubation, were discarded because they fell outside the limits within which the method is applicable.

Calculation of enzymic activity. The activity or apparent concentration of enzyme was found under appropriate conditions to obey the following law: Logarithm of ten times the concentration of liver extract $= 0.00167 \times$ mgm. per cent glycogen loss.

This relationship was used to determine the concentration (in grams per 100 cc. of incubation mixture) of fresh liver arbitrarily selected as the standard which, extracted, would produce the same quantitative glycogenolysis as the unknown. It was then possible to transform this figure for any specific liver into an "α-value" which indicated its amylolytic activity. For the sake of convenience the unit of activity adopted was defined as follows: α-value = 100 $\times$ concentration of the standard liver which would produce an effect equivalent to the unknown.

The final working equation for estimating liver amylase activity then became: α-value of liver amylase per 100 cc. = 10 $\times$ antilog. (0.00167

TABLE 1

Effect of hormones on liver amylase in the fasting state and after feeding glucose (α-value per gram)

	FASTING*		AFTER FEEDING							
			1 hr.		2 hrs.		3 hrs.		4 hrs.	
	Control	Hormone	Control	Hormone	Control	Hormone	Control	Hormone	Control	Hormone
Thyroxin:										
Number of experiments	20	18	13	14	11	11	14	14	17	17
Average	45.3	78.6	45.6	70.7	56.7	64.2	61.9	39.5	43.8	62.5
Median	35.5	67.6	31.7	71.1	43.3	42.0	33.2	38.6	37.2	56.3
Insulin:										
Number of experiments	16	16	12	12	22	24	8	12	8	12
Average	30.5	27.9	49.3	47.1	63.9	26.8	75.8	26.4	43.8	32.0
Median	29.4	22.6	29.7	33.4	45.4	23.6	55.2	24.6	43.3	25.4

* Animals for the thyroxin experiments were fasted only 24 hours; those for insulin 48 hours.

$\times$ mgm. per cent glycogen loss). The significance of the α-value as a measure of amylolytic activity has been discussed elsewhere (1).

Effect of thyroxin. Animals were rendered thyrotoxic by daily subcutaneous injections of 0.04 mgm. of thyroxin (dissolved in dilute alkali) for eight consecutive days. Control mice received a daily injection of an equivalent amount of alkali. In table 1 are shown the averaged results of an assay of 74 livers from thyrotoxic mice, with 75 controls.

Thyrotoxic mice, fasted 24 hours, showed an increase in the α-value of their livers of 74 per cent over those of the controls, if the results were expressed as averages; or a 90 per cent increase when these figures were expressed as medians. After feeding glucose, the median values of the controls remained approximately unchanged; but those of the thyroxin-

injected animals fell, and assumed approximately the same level as the untreated controls.

In table 2 are recorded the results from experiments on the simultaneous determination of liver amylase and glycogen, along with data on the intestinal absorption in the same animals. There was no direct obvious relationship between the level of liver glycogen and the α-value in any single case. However, if the median values of the amylase figures in the control and thyroxin groups were compared with the corresponding average gly-

TABLE 2

Amylase, glycogen and intestinal absorption in thyrotoxic animals and controls after feeding 0.75 gram glucose per 100 gram mouse

TIME AFTER FEEDING		CONTROL			THYROXIN		
		Amylase α-value per gram	Glycogen content, per cent	Intestinal absorption, per cent glucose fed	Amylase α-value per gram	Glycogen content, per cent	Intestinal absorption, per cent glucose fed
1 hour	Number of experiments	4	4	4	4	4	4
	Average	28.5	2.4	52.5	61.4	0.7	74.5
2 hours	Number of experiments	4	8	4	4	7	4
	Average	45.2	3.0	77.7	97.6	1.1	100.0
3 hours	Number of experiments	4	8	4	8	8	4
	Average	35.4	4.6	94.6	36.2	1.0	100.0
4 hours	Number of experiments		4			3	
	Average		3.1			0.1	

cogen content of the liver, it could be seen (fig. 1) that, very roughly, the glycogen level varied inversely to the α-value. Table 2 also demonstrates in a striking manner the inability of thyrotoxic mice to deposit glycogen in the liver in a degree comparable to the normal. That this was not due to failure in absorption of the ingested glucose was shown by the figures on intestinal absorption. Not only did thyroxin-injected mice absorb the glucose fed, but they absorbed it at a rate appreciably faster than the controls.

Effect of insulin. Five units of insulin injected subcutaneously into mice which had been fasted for 48 hours, resulted in convulsions and death within an hour. There was no demonstrable change in the liver amylase of 16 such animals, killed when they developed convulsions, as compared with the 16 controls, killed at the same time. However, if the ani-

mals which had been previously fasted for 48 hours, were fed glucose (0.75 gm./100 gm. mouse) by stomach tube at the same time as the insulin injection, they survived from four to five hours. In these a definite difference could be observed between the control and insulin-injected group.

In table 1 are recorded the α-values of liver amylase at hourly intervals after feeding. The control group was merely fed the measured amount of glucose, while the insulin group received 5 units of insulin subcutaneously at the time of feeding. As compared with the controls, the determinations on insulin-injected animals were much more uniform. Likewise, following glucose-feeding and insulin-injection, the scattering of the points

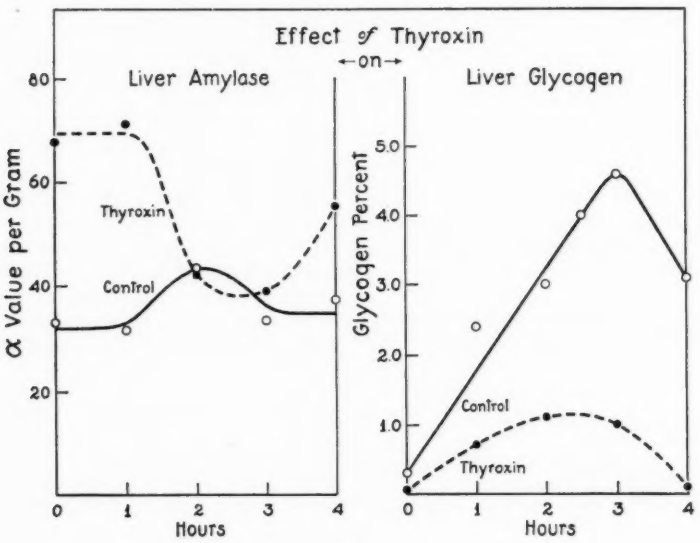

Fig. 1

was decreased. Furthermore, the average and median remained at the starvation level (except, perhaps, at one hour) or fell slightly below it.

The results of glycogen determinations on the livers of similarly treated animals are shown in table 3. In this case the average liver glycogen content of the control animals ran approximately parallel to the α-value, as is shown graphically in figure 2. In the control group, indeed, both glycogen content and α-value of the liver rose, reaching their height at 3 hours after feeding, then declining slightly. After insulin, however, the α-value for liver amylase remained constant (within the experimental error) or fell, while the glycogen content increased but slightly over the fasting level, reaching its height of 0.7 per cent at one hour, then slowly falling.

TABLE 3

Effect of insulin on liver glycogen and intestinal absorption

The mice, previously fasted for 48 hours, were fed glucose (0.75 gm. per 100 gm. mouse) by stomach tube.

	TIME AFTER FEEDING							
	1 hour		2 hours		3 hours		4 hours	
	Control	Insulin	Control	Insulin	Control	Insulin	Control	Insulin
Liver glycogen after insulin:								
Number of experiments	8	16	8	12	8	7	8	14
Average glycogen content of liver in per cent	1.2	0.7	1.9	0.6	2.9	0.3	2.0	0.2
	GLUCOSE FED, MGM.	PER CENT ABSORBED	GLUCOSE FED, MGM.	PER CENT ABSORBED	GLUCOSE FED, MGM.	PER CENT ABSORBED	GLUCOSE FED, MGM.	PER CENT ABSORBED
Intestinal absorption of glucose after insulin:								
Number of experiments	4		4		3		4	
Average	150	58	150	83	135	96	135	98

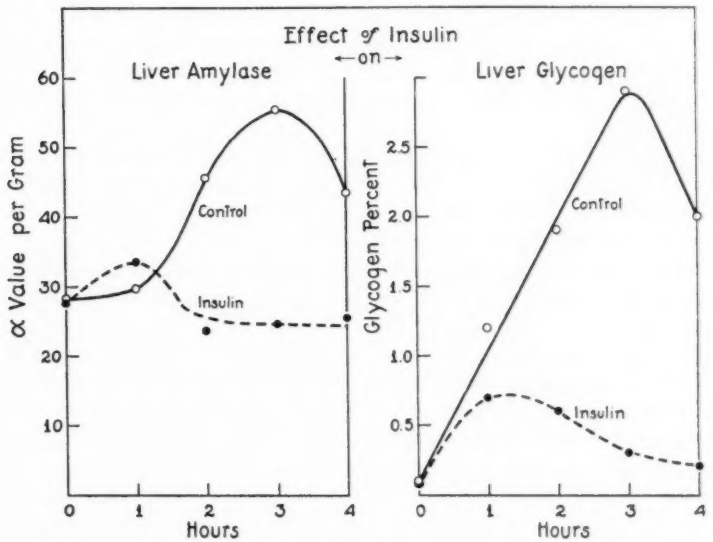

Fig. 2

That this failure to deposit glycogen was not due to failure to absorb the glucose fed is shown by the figures in table 3. These agree well with the control group in table 2.

Effect of epinephrine. After injection of 0.1 cc. of 1–10,000 solution of epinephrine, no demonstrable change was observed in the liver amylase of 48 mice as compared to a similar control group treated in the same manner, but with an injection of saline instead of epinephrine. The injections were given both to starving mice and to mice which had received glucose by stomach tube, but in neither of these groups did the adrenalin-injected mice differ significantly from the controls, although they were killed at intervals between 3 minutes and 2 hours after the injection.

Discussion. As might be expected from the active rôle played by the liver in the homeostasis of glucose (7), the most significant factor revealed by these experiments is the effect of nutritional state upon the amylase of the liver. Indeed, the results strongly suggest that increased enzyme activity regularly accompanies the storage or release of liver glycogen. It has been impossible to prove that changes in enzyme activity actually precede the shift of carbohydrate, but this remains a distinct possibility.

As regards the influence of hormones upon the apparent concentration of this enzyme in the liver, the results are significant only when the nutritional effect can be minimized. This condition is best fulfilled in the fasting animal. Under this condition, the marked elevation in enzymic activity of thyrotoxic livers is in striking contrast to the low level of the control livers. Feeding obscures this difference, because the administration of food may elevate amylase activity maximally. Indeed, the definite alteration of amylase activity found in the tissues of our thyrotoxic animals may well be intimately connected with the low liver glycogen found in such animals.

The effect of insulin is signalized by its preventing the postprandial increase of enzymic activity. Here, again, no hormone effect would have been observed had not the state of nutrition been rigidly controlled. Both results serve to emphasize the interrelation of endocrine activity with the nutritional status of the organism. They indicate further that the activity of enzymes within cells is intimately concerned with fluxes of carbohydrate metabolism.

Other authors have commented upon liver amylase activity in mammals. Macleod (8) states that fasting elevates enzymic activity in the dog liver. This would be true, in the light of the experiments here reported, only during the earlier stages of glycogen depletion. Reid, Quigley, and Meyers (9) report that normal dogs show an increase in liver amylase activity following the administration of insulin. This finding is at variance with our results in mice. Here, again, it seems likely that the discrepancy is based upon inadequate control of glycogen turnover. Under the con-

ditions of our experiments, both insulin and thyroxin prevented the storage of glycogen in the liver, despite adequate absorption of glucose from the intestine.

The wide range of variability in liver amylase activity deserves comment. In view of the relative constancy of the blood sugar, it seems logical to assume that there must exist in the organism a rather labile mechanism which reacts readily to minor changes in the environment. By this means, fluctuations in blood sugar are avoided by vicarious fluctuations in the accommodating apparatus. This rôle of the liver in the homeostasis of blood sugar has been well described by Mann and Magath (10) and by Cannon (7). It seems reasonable to assume that the wide variations in liver enzyme activity may be associated with the homeostatic function.

SUMMARY

A method is described for estimating the activity of liver amylase which permits one to study the effect of hormones upon the apparent concentration of the enzyme in mice. In such experiments, the nutritional state of the animals had to be rigidly controlled because postprandial elevation of amylase activity was large and widely variable. The variability was minimized by the administration of insulin, which depressed the activity of the enzyme to that of the fasting condition. Thyroxin produced a marked increase of enzymic activity in the fasting state; but immediately after feeding thyrotoxic animals, the amylase dropped temporarily. Epinephrine showed no obvious effect in fasting or fed animals.

Liver glycogen was found to be low in both insulin- and thyroxin-treated animals. In general, variations in the glycogen concentration of the liver tended to increase the apparent concentration of liver amylase, whereas in a more steady state of glycogen balance, the activity of amylase remained low or constant. These results suggest that increased enzyme activity regularly accompanies the storage or release of liver glycogen.

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THE PHOSPHOLIPID CONTENT OF LIVER, SKELETAL MUSCLE AND WHOLE BLOOD AS AFFECTED BY THYROXINE INJECTIONS

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There are two general theories of phospholipid function which are useful in explaining the relatively constant amount of phospholipid found in a given tissue. One holds that these substances participate in fatty acid transport and combustion, and is said to be supported by experiments showing replacement of phospholipid fatty acids by food fatty acids. The second theory holds that tissue phospholipids are structural portions of protoplasm, concerned with the general metabolic activity of the cell, but not with any specific metabolic process; offered in support of this theory are observations indicating a parallelism between metabolic or functional activity and phospholipid content.

The validity of these theories might be tested by studying the effect of thyroxine administration on the phospholipid content of different tissues, for such treatment increases both metabolic activity and the rate of fat combustion. The present report deals with the changes in the phospholipid content of liver, skeletal muscle and whole blood produced by thyroxine injections. Changes in non-phospholipid fatty acid content are also recorded since, normally, definite quantities of these substances are associated with the phospholipids.

EXPERIMENTAL MATERIALS AND METHODS. *Animals.* New Zealand White strain rabbits, of both sexes, 8 to 11 months of age and weighing 3 to 4 kilos, were kept in the laboratory 3 to 5 months before use. Since preliminary work demonstrated that effects of thyroxine on tissue lipids are modified by environmental temperatures, these experiments were carried out with the temperature of the animal quarters maintained at 22°C. ±2.5°.

General procedure. One group of rabbits received daily intravenous injections of crystalline thyroxine[1] for 10 days; the daily doses varied from 0.4 to 1.0 mgm.; the total amounts injected varied from 4 to 8.2 mgm.

[1] This crystalline thyroxine was supplied through the generosity of E. R. Squibb and Sons, New York.

Such treatment produced decreases in body weight, ranging from 8 to 33 per cent. On the twelfth day, following a 24 hour fast, the rabbits were stunned and bled to death. The liver of each animal was quickly removed, freed from the gall bladder and adherent blood, and weighed to 0.1 gram; a weighed portion of the left lobe of the liver was used for lipid analyses. A portion of the biceps femoris muscle, removed from the left rear leg and freed from adherent connective tissue was also used for lipid analyses.

The changes in whole blood lipids were followed only in female rabbits. Prior to the initial thyroxine injection, and every three days thereafter, 10 cc. of blood were obtained by heart puncture. (A 24-hour fast preceded each heart puncture.) On the last day of the experiment, 30 cc. were withdrawn so as to provide material for both whole blood and plasma analyses.

The tissues of animals injected with thyroxine were compared with those of normal control rabbits.

TABLE 1

The effects of thyroxine injections on the water content of liver and skeletal muscle

NO. OF ANIMALS IN GROUP	SEX	TREATMENT	WATER CONTENT					
			Liver			Skeletal muscle		
			Maximum	Minimum	Mean	Maximum	Minimum	Mean
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
8	♀	Thyroxine injected	75.5	70.2	72.0	72.7	69.0	71.4
6	♀	Normal control	71.6	68.2	69.7	73.6	72.9	73.3
7	♂	Thyroxine injected	74.3	69.4	71.6	71.4	68.5	70.3
9	♂	Normal control	70.3	68.5	69.3	74.2	71.9	72.9

Analytical procedure: Estimation of water content of blood and tissues. Weighed samples of liver, skeletal muscle, whole blood or plasma, in tared beakers, were covered with 10 cc. of a mixture of three parts of absolute ethyl alcohol and one part of absolute ethyl ether. The alcohol ether mixture was removed on the steam bath and the beaker and residue dried in a hot air oven, 95 to 96°C.; constant weight was usually obtained in 48 to 60 hours. The experimental error of this method was ± 0.5 per cent. The results of these determinations are summarized in table 1 and figure 1.

Thyroxine injections definitely increased the water content of liver (table 1). Livers of normal male and female rabbits contained 69.3 and 69.7 per cent of water (average), respectively, while the livers of thyroxine injected males and females contained 71.6 and 72 per cent, respectively.

However, thyroxine injections decreased the water content of skeletal muscle (table 1). Skeletal muscles of normal male and female rabbits contained on the average, 72.9 and 73.3 per cent water, respectively; the same tissues of thyroxine treated animals averaged 70.3 and 71.4 per cent.

Since these variations in water content would of themselves produce relative decreases in liver and increases in muscle lipid content, all lipid data except those of whole blood have been calculated and recorded as per cent of dry tissue.

Figure 1 shows that thyroxine injections do not significantly change the water content of whole blood. The terminal increase in blood water, occurring in both normal control and treated rabbits, must have been caused by a factor other than thyroxine—possibly by repeated withdrawal of blood.

Extraction of tissue lipids. Whole blood and plasma lipids were extracted by the Bloor (1) procedure, 5 cc. of whole blood or plasma being extracted with 100 cc. of the alcohol ether mixture.

Samples of liver and skeletal muscle were hashed with a sharp scissors and scalpel, immediately after extirpation. Then 2 to 3 gram samples of liver or 3 to 4 gram samples of muscle were rapidly weighed to 1 mgm., transferred to a small mortar, thoroughly ground with approximately 10 grams of sand (hydrochloric acid washed, alcohol and ether extracted), transferred to a 125 cc. Erlenmeyer flask, and extracted successively with four 30 cc. portions of the boiling alcohol and ether mixture. These extracts, filtered through fat free filter paper, were combined, cooled, made up to 100 cc. volume and stored in a cool dark cupboard. This method gave complete extraction, since boiling the final tissue and sand residue with a fifth portion of alcohol and ether failed to remove determinable quantities of the different lipids.

Phospholipid fatty acids. Phospholipids were determined by the oxidative procedure of Bloor (2); as used, the experimental error was ± 1.5 per cent. The results are recorded in terms of phospholipid fatty acids—considering the fatty acids as 73 per cent of the total phospholipid. In addition phospholipid phosphorus was determined on the phospholipid precipitate, using a modification of the Fiske and Subbarow (3) method. The latter results are not recorded since the ratio of phospholipid to phospholipid phosphorus was the same in normal and thyroxine injected rabbits, varying from 24 to 25:1.

Non-phospholipid fatty acids. Total fatty acids and cholesterol were determined by the Bloor (4) procedure—the results being expressed in equivalents of oleic acid. Total fatty acids were calculated by subtracting the oleic acid equivalent of the total cholesterol[2] (determined by the Yasuda (5) modification of the Okey (6) procedure) from the total fatty acid and cholesterol values. Non-phospholipid fatty acids were calculated as the difference between the total fatty acids and the phospholipid fatty acid values, and are thought to be principally neutral fat in origin.

[2] As Sakai (7) and Furukubo (8) had previously observed, the total cholesterol content of both liver and skeletal muscle was increased by thyroxine injections.

RESULTS. As the data of table 2 show, *thyroxine injections decreased the liver phospholipid content.* The livers of normal male and female rabbits contained, on the average, 8.48 and 8.27 per cent of phospholipid fatty acids; livers of thyroxine injected males and females averaged only 6.58 and 6.72 per cent, respectively.

In contrast to this, *thyroxine injections increased the phospholipid content of skeletal muscle* (table 2). Whereas the biceps femoris muscle of normal males and females contained an average of 1.88 and 1.84 per cent of phospholipid fatty acids, that of thyroxine treated rabbits averaged 2.33 and 2.36 per cent for respective sexes.

TABLE 2

The effects of thyroxine injections on the phospholipid fatty acid content of liver and skeletal muscle

NO. OF ANIMALS IN GROUP	SEX	TREATMENT	PHOSPHOLIPID FATTY ACIDS (PER CENT OF DRY TISSUE)					
			Liver			Skeletal muscle		
			Maximum	Minimum	Mean	Maximum	Minimum	Mean
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
8	♀	Thyroxine injected	7.56	5.83	6.72	2.56	2.16	2.36
6	♀	Normal control	9.20	7.11	8.27	1.96	1.64	1.84
7	♂	Thyroxine injected	7.09	5.73	6.58	2.60	2.15	2.33
9	♂	Normal control	9.87	7.45	8.48	2.04	1.68	1.88

The data of table 3 show that *thyroxine injections increased the non-phospholipid fatty acid content of liver.* This increase is much larger in females than in males—350 per cent in females (from 1.31 to 5.87 per cent), and only 33 per cent in the males (from 3.36 to 4.4 per cent).

Table 3 also shows that *thyroxine injections decreased the non-phospholipid fatty acid content of skeletal muscle.* The muscles of normal males and females averaged 1.46 and 2.27 per cent of non-phospholipid fatty acids; those of thyroxine treated rabbits averaged 1.19 and 1.44 per cent, respectively.

As figure 2 shows, thyroxine injections increased whole blood phospholipids—from an initial average of 152 mgm. of phospholipid fatty acids to 191 mgm. (per 100 cc. of blood) at the end of treatment. Plasma phospholipids were also increased after thyroxine injections, but the size of this increase was not sufficient to account for the entire whole blood change.

Figure 3 shows that the non-phospholipid fatty acid content of whole blood increased in both normal controls and thyroxine injected rabbits. As Horiuchi (9) observed, the increase in the control group may have been due to repeated withdrawal of blood. Since the increase in the thyroxine group was double that of the controls, it seems probable that it

is partially due to thyroxine injections. This change occurred almost exclusively in the plasma.

TABLE 3

The effects of thyroxine injections on the non-phospholipid fatty acid content of liver and skeletal muscle

NO. OF ANIMALS IN GROUP	SEX	TREATMENT	NON-PHOSPHOLIPID FATTY ACIDS (PER CENT OF DRY TISSUE)					
			Liver			Skeletal muscle		
			Maximum	Minimum	Mean	Maximum	Minimum	Mean
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
8	♀	Thyroxine injected	10.61	1.60	5.87	3.13	0.54	1.44
6	♀	Normal control	1.95	0.43	1.31	3.26	1.47	2.27
7	♂	Thyroxine injected	7.05	2.18	4.40	1.65	0.72	1.19
9	♂	Normal control	4.46	2.02	3.36	1.93	1.07	1.46

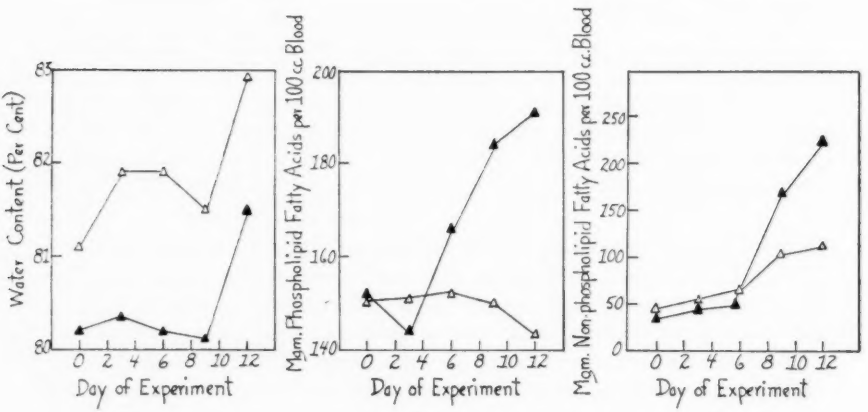

Fig. 1 Fig. 2 Fig. 3

Fig. 1. The average effect of thyroxine injection on the water content of whole blood: ▲ = thyroxine injected, △ = normal control.

Fig. 2. The average effect of thyroxine injection on the phospholipid fatty acid content of whole blood: ▲ = thyroxine injected, △ = normal control.

Fig. 3. The average effect of thyroxine injection on the non-phospholipid fatty acid content of whole blood: ▲ = thyroxine injected, △ = normal control.

DISCUSSION. The fact that thyroxine injections decrease the phospholipid content of liver and at the same time increase that of skeletal muscle is of interest in considering phospholipid function.

The observations of Kulbs (10), Bloor (11), Sorg (12) and Bloor and Snider (13), indicating a parallelism between phospholipid content of dif-

ferent muscles and their physiological activity, are said to support the general metabolic theory of phospholipid function. The increase in skeletal muscle phospholipids following thyroxine injections may also be interpreted as favoring this theory. However, the decrease in liver phospholipids is not easily explained on this basis.

It is possible to explain the present liver changes by assuming different functions for liver and skeletal muscle phospholipids. As Sinclair (14) has suggested, the phospholipids of liver may be concerned with the intermediary metabolism of fatty acids as well as certain general metabolic processes, while in muscle, phospholipids may have only a general metabolic function.

The present changes in liver and muscle may also be explained by assuming that the phospholipids of both tissues participate in fat metabolism. In the liver, the phospholipids may be primarily concerned with desaturation of the fatty acids in preparation for combustion in such tissues as muscle. When the fatty acid requirements of these tissues are markedly increased, as by thyroxine injections, a more rapid breakdown of liver phospholipids may occur. Or liver phospholipids may be released into the blood stream and assist in fatty acid transport. The increase in whole blood phospholipids supports this possibility. On the other hand, if fatty acids in muscle are burned in phospholipid combination, the amount of phospholipid in the tissue may be proportional to the amount of fatty acid burned. Such a condition might also explain the parallelism between metabolic activity and phospholipid content.

The increase in liver and decrease in muscle non-phospholipid fatty acids, together with the disappearance of adipose tissues (15) following thyroxine injections, are indicative of a disturbance in fat metabolism. Whether the phospholipid changes result from this disturbance or are only coincidental is a matter for further experimentation.

SUMMARY

Injection of thyroxine into rabbits of both sexes produced the following tissue lipid changes; the phospholipid fatty acid content of liver decreased, while the non-phospholipid fatty acid content increased; in skeletal muscle, the reverse changes occurred—phospholipid fatty acids increased and non-phospholipid fatty acids decreased; in blood, both phospholipid and non-phospholipid fatty acids increased.

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PROOF OF A HUMORAL CONTROL OF INTESTINAL SECRETION

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Ever since Thiry (1864) devised his method for the collection of pure intestinal juice there has been considerable controversy concerning the mechanism involved in the production of this secretion. Beginning with Thiry much evidence has accumulated to indicate that food in the GI tract will increase the secretion from an isolated loop of small intestine. These data can be interpreted as the result of either a humoral or a reflex action, or a combination of both. Other investigators have been led by their observations to the conclusion that food in the GI tract has no influence upon the secretion from isolated loops. From the fact that still other workers have noticed a decreased secretion following a meal it has been proposed that the mesenteric nerves carry fibers which inhibit secretion. This idea receives support from the work of Moreau (1868) who found that denervation of an intestinal loop resulted in a greatly accelerated production of intestinal juice in that loop. The literature on the subject up to 1928 has been fully reviewed by Babkin (1927) (1928) who states that probably there is a dual, nervous-humoral, control of this secretion. The work since 1928 has not been very extensive. Cajori (1933) has recently observed that feeding may be followed by a diminution in intestinal secretion. W. N. Boldyreff (1928) found that food had no effect. J. W. Boldyreff (1928) reported that feeding bones resulted in augmented secretion which, however, did not appear until the animal had been fed for several days.

In order to differentiate between the humoral and the extrinsic nervous factors, it is necessary to make certain that all of the extrinsic nerves are cut. This has been accomplished in the present series of experiments. The technic was suggested by Ivy and Farrell's (1925) brilliant success in demonstrating a humoral control of pancreatic and gastric glands by their method of transplantation of the secreting structures to the submammary surface of the skin in recently lactating female dogs. Ivy, Farrell and Lueth (1927) had indeed transplanted loops of intestine in this manner and had noted that "normal" intestinal juice was secreted, but this work was overlooked until after several dogs of the present series had been operated.

Ivy and his associates presented no evidence bearing on the control of the intestinal glands, and, apparently, they have not returned to the subject. The general technic adopted here proved to be very similar to that described and pictured in the paper from Ivy's laboratory just referred to. Thus far only loops from the jejunum, starting just below the ligament of Treitz, have been studied. The plan was to make a transplant of an intestinal segment, as Ivy and Farrell describe for the transplantation of a gastric pouch, then after sufficient time to permit of the establishment of a good anastomosis with the mammary blood vessels, to cut off the mesenteric pedicle. If the loop should survive this procedure, remain in good functional condition, and should respond to feeding by producing more juice we should have proof of the humoral control, because extrinsic nervous control would be impossible.

TABLE 1

DOG NO.	WT.	DATE OF OPERATION		TIME BETWEEN OPERATIONS	REMARKS
		1st stage	2nd stage		
	kgm.			*weeks*	
II	18	12–29–30	4– 2–31	13	"Horseshoe" loop, 20 cm. upper jejunum
III	15	3–12–31	6–13–31	13	"Horseshoe" loop, 20 cm. upper jejunum
IV	25	6–17–31	12– 1–31	24	"Horseshoe" loop, 15 cm. upper jejunum
V	13	1–27–32	9– 6–32	32	Two 10–12 cm. loops, bilateral, upper jejunum
VI	20	2– 8–32	9– 6–32	30	Two 10–12 cm. loops, bilateral, upper jejunum
VII	18	2–13–33	2–13–34	52	Two 10–12 cm. loops, bilateral, middle jejunum
VIII	27	3– 1–33	2–13–34	50	Two 10–12 cm. loops, bilateral, lower jejunum

EXPERIMENTAL. The transplantation experiment was done as a demonstration to medical students in November, 1929 and November, 1930. One of these was quite successful and was used by a group of students for study of the enzymes contained in the intestinal juice collected. Later it was used by two of us for further study of the secretion after the first stage operation. Still later the loop became too small for successful use and the dog was discarded. The first dog to come through the second stage operation successfully is described below as dog II.

Under ether-morphine anesthesia segments of the jejunum were transplanted to the submammary surface in the abdominal wall of recently lactating dogs leaving the mesenteric nerves and blood vessels intact. Throughout the paper this is referred to as the *first stage* operation. After the establishment of a sufficient collateral circulation from the mammary and epigastric vessels, the *second stage* operation was performed in which the mesenteric pedicle was doubly ligated and cut. The peripheral end was brought out and the stump sutured to the rectus fascia. The number

of dogs with a brief description of the type and size of loops is given in table 1. The "horseshoe" loop is a single one which lies in a semicircle with its diameter at right angles to the long axis of the body. Because of difficulty in inserting catheters to the end of such loops it was found more satisfactory to transplant two straight but shorter loops, one on each side of the midline.

For collection of juice smooth catheters were always inserted into the loops to furnish a constant mechanical stimulus. This is necessary in order to obtain an appreciable amount of juice. The details of the technical procedure are to be found in another paper (Pierce, Nasset and

Fig. 1. Showing adjustable stall for dog while intestinal juice is collected. The harness gives support to the animal and holds collecting vessel and catheters securely.

Murlin, 1935). The dog was set up in a stall (fig. 1) with suitable harness for holding the vessel in place and for affording some support to the animal in order to avoid excessive fatigue.

Results. The crucial experiment to prove humoral control of the intestinal secretion consists *a*, in showing that more juice is secreted after a meal than while the dog is fasting, and *b*, that a greater quantity of one or more enzymes is secreted, the transplanted segment being in good condition in both nutritive states. Of the two tests the latter is more important because the former (more juice) might well be due merely to a passage of water after food and therefore have no specific relation to the digestive processes. It is characteristic of a great many investigations on digestive

juices that the volume has been the chief, if not the only measurement employed.

Volume of secretion. The evidence for greater volume after food was obtained in the first stage with dogs II, III and IV. Dog V showed scarcely any change whereas with dogs VI, VII and VIII there was an apparent inhibition (table 2). The grand average for the seven dogs,

TABLE 2

Volume of intestinal juice

DOG NUMBER	FASTING			FOOD		
	Number of collections	Number hours averaged each	Average volume per hour	Number of collections	Number hours averaged each	Average volume per hour
			After first stage operation			
			cc.			cc.
II	5	7	2.4	5	7	3.4
III	4	7	2.2	4	7	4.7
IV	4	7	2.6	4	7	3.4
V	8	7	3.0	5	7	3.1
VI	4	7	6.1	7	7	5.4
VII	9	7	1.3	8	7	1.2
VIII	14	7	6.8	10	7–8	4.3
	Total 48	Grand av. 3.5		Total 43	Grand av. 3.6	
			After second stage operation			
II	3	7	1.6	3	7	1.5
III	2	7	1.4	1	7	3.4
IV	12	3	3.3	9	4	4.2
	3	7		2	7	
V	17	7	2.0	16	7	2.2
VI	18	7	4.9	18	7	10.3
VII	6	7	0.5	7	7	1.1
VIII	8	7	4.1	7	7	5.6
	Total 69	Grand av. 2.5		Total 63	Grand av. 4.0	

first stage in 48 collections, fasting, is 3.5 cc. per hour; for 43 collections after food it is 3.6 cc., an increase of only 3 per cent. The grand average for the second stage, fasting, is 2.5 cc. per hour, and, after food, 4.0 cc., an increase of 60 per cent. The effect of food after the second stage by this showing is twenty times as great as in the first stage. This result, however, is largely attributable to dog VI, which was one of the best producers of juice at all times and particularly after food in the second stage. Omitting this dog the percentage increase following denervation is only 20 compared

with 60. This is not to say that dog VI should be ruled out, but only to say that an average result with so small a number of dogs is not so accurate as is the simple statement that in all dogs but one after the second stage there was a greater volume of juice after food than in fasting. The fact that the increase caused by food was a substantial one after the segments were denervated *proves that the path of influence exerted by the food is humoral.* The change in response to a meal brought about after the second operation shows that in some animals the nervous inhibition of secretion is quite dominant. The erratic behavior of the animals in the first stage indicates very clearly the reason for the divergence of opinion, expressed in the literature, concerning the effect of a meal upon the secretion of the intestine. Had our observations been limited to dogs II, III, IV and V, first stage, we might have said that, without exception, the ingestion of a meal was followed by an increased production of intestinal juice. On the other hand, the results obtained only from the other three dogs would have forced us to the opposite conclusion. Dogs VI and VIII recall in many details the rôle of inhibitory nerves proposed by Falloise (1904) and Savitsch (1922). In the first stage dog VI, though a good producer, secreted in the early hours of collection less juice after food than in fasting; while after the nerves were cut the increase caused by food was more than 100 per cent. The hour to hour average of secretion rate in this animal and dog VIII is shown in figure 2. It is obvious that the mechanical stimulus of the catheters in the first stage produces much more juice in the early hours than later, and also more *in these hours* during inanition than after food; later in the day the response becomes more nearly the same in the two conditions. In the second stage the response is much greater throughout the day in dog VI after food than in fasting.

Enzyme production. The quantitative methods for study of enzyme production adopted in this investigation are described in another paper (Pierce, Nasset and Murlin, 1935; see also 1932). The effects vary considerably from dog to dog and from day to day in the same dogs, as one would expect. Hence a large number of collections of juice and detailed analyses for the results of digestion by sucrase, peptidase (erepsin), lipase, and the amylase-maltase system obviously were necessary. For comparative purposes the amount of enzyme present is expressed as the 48 hour enzymic activity of an hour's average secretion. A general summary of the effects of food both before and after complete isolation of the loops is presented in table 3. The total number of collections and analyses is 189.

The general trend of the results is unmistakable. With the exception of sucrase in dogs II, IV and V, peptidase in IV and lipase in V, food in the alimentary tract increases the total amount of enzymes produced throughout the day in the second stage. It should be mentioned here that in most experiments with dog IV the meal was given at noon following a prelimi-

nary 3 hour fasting period. The juice collected during the 4 hours subsequent to feeding was compared as to quantity and quality with that collected before the meal. It developed later, with other dogs, that the maximum secretion after a meal is reached only after 3 or 4 hours (fig. 2) and therefore this method of comparison makes dog IV appear exceptional in table 3, whereas the difference is due to a different mode of collection.

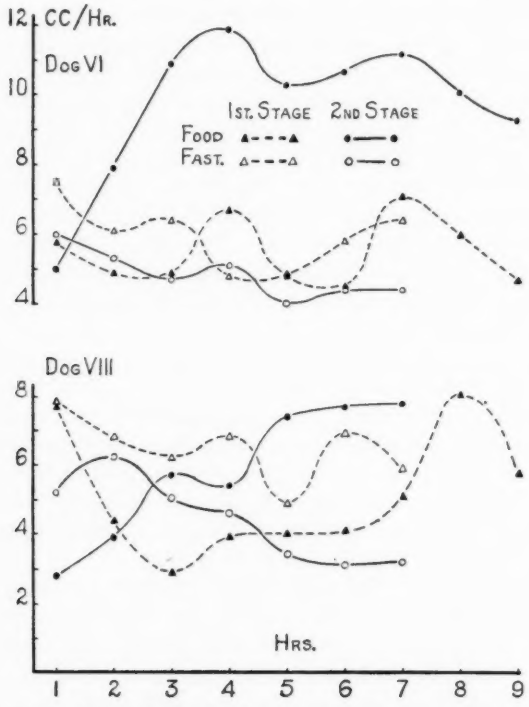

Fig. 2. Secretion of intestinal juice by jejunal segments. The data for feeding experiments are arranged so that the abscissa shows the time elapsed between the meal and subsequent hourly collections. The points on the fasting curves show the time from the beginning of collection.

Moreover, the first portion of juice may contain a higher concentration of enzymes than following portions.

It should be noted further that the erratic and equivocal results in the second stage are to be found in the first four dogs. The data in table 3 show that the average hourly production of the 4 enzymes studied is much less in the second stage than in the first which may mean that the circulation was incompetent. The length of time allowed to elapse between the

TABLE 3

48-hour enzymic activity of an hour's average secretion

Summary of 189 collections and analyses

DOG	EXPERIMENT	AFTER FIRST OPERATION						AFTER SECOND OPERATION					
		No. collections*	Sucrase, invert sugar	Amylase-maltase, glucose	Peptidase, amino N	Lipase, fatty acids	Average hourly vol.	No. collections*	Sucrase, invert sugar	Amylase-maltase, glucose	Peptidase, amino N	Lipase, fatty acids	Average hourly vol.
			mgm.	*mgm.*	*mgm.*	*mgm.*	*cc.*		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*cc.*
II	Food	5	896	1081	91	273	3.4	2	87	748	80	88	1.5
	Fast	3	759	761	76	277	2.4	1	120	580	16	52	1.6
	Food effect		+18%	+42%	+20%	−1%	+42%		−31%	+29%	+400%	+69%	−6%
III	Food	3	1273	1229	90	171	4.7	1	265		15		3.4
	Fast	3	890	914	66	125	2.2	2	60	300	9		1.4
	Food effect		+43%	+34%	+36%	+39%	+114%		+342%		+67%		+143%
IV	Food	3	1285	1274	87	204	3.4	10	317	843	37	60	4.2
	Fast	3	955	1006	69	183	2.6	16	430	838	40	54	3.3
	Food effect		+35%	+27%	+26%	+11%	+31%		−26%	+1%	−8%	+15%	+27%
V	Food	5	1493	1453	146	328	3.1	16	701	904	57	125	2.2
	Fast	7	1423	1482	115	262	3.0	16	748	880	54	137	2.0
	Food effect		+5%	−2%	+27%	+25%	+3%		−6%	+3%	+7%	−9%	+10%
VI	Food	7	1673	2169	121	268	5.4	16	1682	2063	130	375	10.3
	Fast	3	2359	2614	178	493	6.1	16	1402	1595	90	281	4.9
	Food effect		−29%	−17%	−32%	−46%	−11%		+20%	+29%	+44%	+33%	+110%
VII	Food	8	199	491	47	60	1.2	3	509	635	94	217	1.1
	Fast	9	497	707	52	84	1.3	3	286		32		0.5
	Food effect		−60%	−21%	−10%	−29%	−8%		+78%		+194%		+120%
VIII	Food	8	537	882	98	101	4.3	6	548	1054	96	184	5.6
	Fast	9	1477	1744	216	176	6.8	5	395	945	87	181	4.1
	Food effect		−64%	−49%	−55%	−43%	−37%		+39%	+12%	+10%	+2%	+37%

* Number of experiments from which the average for the enzyme values was derived.

two operations (table 1) bears some relation to the activity of the isolated segments in the second stage; the segments having the longest time between transplantation and ligation and section of the mesenteric pedicle apparently have the best chance of establishing a competent collateral circulation. In fact, the secretory activity of the loops in dogs II and III declined to such an extent in the second stage that they had to be discarded. For this reason we have very few experiments with these animals in the second stage. The behavior of dog V, however, cannot be explained on this basis. A contrast is afforded in the first stage by dogs VI, VII and VIII in which the inhibition, initiated by food and doubtless exerted through the extrinsic nerves, is very striking. The average of only a small number of experiments (as with dogs II, III and VII) gives in some of the columns very large percentage increases. The average of a large number (as with dogs V, VI and VIII) moderates the effect. This is not an arithmetic result solely; it signifies rather that on certain days, owing to apparently specific effects, not yet fully elucidated, a certain enzyme is produced out of all proportion to the others. (Some experiments attempting to disclose evidence of specificity will be found a little further along.)

Humoral control is proved beyond a doubt by the fact that the digestion of food is accompanied by a larger volume of juice as well as a greater enzyme production from the second stage loop. This is obvious from inspection of table 3 and neither a general average for the several dogs nor a weighted average would make the evidence more convincing. As a rule the several experiments on any one dog were consistent. Dog IV, for example, usually produced a more concentrated juice while fasting than after food. The total effect of food on enzymes therefore depends on the fact that a greater volume of juice was produced. With dog VI, on the other hand, there is no consistency as between the experiments before and after denervation, but a marked consistency within each of these groups. Previous to denervation, the loops produced a juice after food which was less concentrated with respect to enzymes than in fasting; also the dog produced less juice. Hence the negative or inhibitory effect shown in table 3 is attributable to the impoverishment both in quality and in quantity. After denervation impoverishment in quality continues but it is more than compensated by the 110 per cent increase in quantity.

Specific effects. This particular study, being devoted primarily to the accumulation of evidence for a humoral control of the intestinal secretion, contains very few data on the comparative effects of different foods. The food which gave best results always contained a liberal amount of protein. Dogs IV and VI proved to be particularly good producers after complete isolation of the loops. They were used therefore to test the effects of some

specific substances and different meals. Table 4 exhibits the effects for dog IV in the second stage.

Vitamin B (Harris) placed in the stomach seemed to stimulate positively the production of enzymes in the afternoon juice as compared with the forenoon collection (fasting). A meal of starch and sugar on two occasions depressed the production of all enzymes. Cream, as judged from one trial, exerted an inhibitory effect in general, up to four hours. The dose of purified secretin was at least 50 times that required to cause a decided increase in pancreatic secretion and yet the result on the intestine was relatively very small.

TABLE 4

Attempts at showing some specific effects in dog IV (48-hour enzymic activity of an hour's average secretion)

MEALS	DAYS AFTER OPERATION	A.M. CONTROL (3 HOURS)				P.M. EXPERIMENTAL (4 hours)			
		Sucrase, invert sugar	Amylase-maltase, glucose	Peptidase, amino N	Lipase, fatty acids	Sucrase, invert sugar	Amylase-maltase, glucose	Peptidase, amino N	Lipase, fatty acids
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	43	118	622	26	32	26	268	8	18
2	49	589	876	52	59	387	599	43	47
3	50	349	787	48	94	145	657	38	66
4	62	120	429	27	32	156	392	30	33
5	63	218	256	31	40	208	322	34	46
6	64	603	746	67	128	66	356	28	36
7	69	164	362	24	31	278	535	41	66

Meals at noon by stomach tube: 1, 185 grams sucrose + 185 grams starch made to 500 cc. 2, 500 cc. warm H_2O. 3, 93 grams sucrose + 93 grams starch made to 500 cc. 4, 150 cc. 0.4% HCl. 5, 50 mgm. purified secretin (intravenously). 6, 1 pint heavy cream. 7, 2 grams vitamin B (Harris).

It is obviously important for comparison of the effects of different foods that the animal be tested for fasting and food effects without the lapse of too much time between to be reasonably certain that the condition of the loop has not changed. With dog VI the collections were made on adjacent days. Table 5 presents some results with this dog. These are typical experiments. In the first stage this dog regularly showed an inhibition of secretion and decreased concentration of enzymes after food (table 2). The effect is seen in table 5 to be much greater at about 100 days after the operation than at 50 days. In the second stage the effect changes promptly to increased production of both fluid and specific constituents. Quite possibly at 6 and 7 days the paralytic secretion had not yet subsided. It certainly was terminated before 105 days, at which time the maximum effect on peptidase secretion was obtained. The kitchen scraps contained

TABLE 5

Attempts at showing some specific effects in dog VI (48-hour enzymic activity of an hour's average secretion)

DAYS AFTER OPERATION	EXPERIMENT	Sucrase, invert sugar	Amylase-maltase, glucose	Pepidase, amino N	Lipase, fatty acids
		1st stage			
		mgm.	*mgm.*	*mgm.*	*mgm.*
50	Food (kitchen scraps)	3629	4673	241	790
49	Fasting	4822	4927	348	929
	Food effect	−25%	−5%	−31%	−15%
105	Food (hamburg)	2012	3109	138	345
106	Fasting	3665	4123	292	915
	Food effect	−45%	−25%	−53%	−62%
		2nd stage			
6	Food (kitchen scraps)	2940	3312	204	471
7	Fasting	2136	2879	139	454
	Food effect	+38%	+15%	+48%	+4%
105	Food (kitchen scraps)	3213	5219	419	910
104	Fasting	2875	3030	170	647
	Food effect	+12%	+72%	+146%	+41%
111 and 113	Food (kitchen scraps)	2722	3131	220	199
112	Fasting	1374	1400	130	269
	Food effect	+98%	+124%	+69%	−26%
133	Food (beef heart)	2164	4622	327	351
132 and 134	Fasting	2845	3269	165	489
	Food effect	−24%	+41%	+98%	−28%
139 and 141	Food (beef heart)	3075	4093	287	600
140	Fasting	3083	3464	133	379
	Food effect	±0%	+18%	+116%	+58%
161	Food (hamburg)	4555	4509	226	1100
160 and 162	Fasting	3721	3879	262	915
	Food effect	+22%	+16%	−14%	+20%
167 and 169	Food (hamburg)	4226	4182	234	1084
168	Fasting	3433	4133	227	910
	Food effect	+23%	+1%	+3%	+19%

a liberal precentage of meat, some bread and other cereal products and quite a percentage of butter and other fats. The dog was fed regularly on this diet at 3 p.m. daily except on collection days when she was fed either immediately before or after the collection period.

The effect of this food was quite variable throughout, as regards sucrase, but was progressive as regards amylase. This recalls the well-known effect of a starch diet in man on salivary amylase. Peptidase increased to a maximum and then declined, as did lipase. Beef heart restored the effect on peptidase but the effect on amylase fell off and continued to fall on ground beef steak, while the effect on lipase was restored by beef heart and continued at a fair level on ground beef steak. The indications of specific effects are found chiefly in the amylase column on kitchen scraps and in the peptidase column on beef heart which was free of visible fat. The table exhibits some inconsistencies, however, which we cannot explain. To prove or disprove the presence of specific effects of food on intestinal secretion beyond a doubt will require considerably more data. This series of observations on dog VI confirms J. W. Boldyreff's single experiment showing the major effect of food on composition of the secretion after several days of feeding. The effect in dog VI, however, was obtained *after complete denervation.*

Periodic secretion. There is evidence in this work of alternate periods of comparative quiescence and heightened secretory activity, termed by W. N. Boldyreff (1928) the "periodic secretion" of the intestine. Figure 2 shows the behavior of two dogs in this regard. These curves are averages based on 2 to 16 observations for each hour, hence do not exhibit the greatest variations shown in single experiments. The periods of greatest secretory activity may be two to four hours apart, which interval is about twice as long as reported by W. N. Boldyreff. Orbeli (1922) noted that denervation increased the interval between periods of activity. In the present series there appears to be no remarkable difference in frequency effected by the second stage operation. Bickel and Wagner (1934) found that the secretion of juice from an isolated loop of ileum in the human was diphasic. The first phase began immediately (2–5 min.) after the ingestion of a meal and continued for 3 to 4 hours. This was followed by a slowing or a cessation of secretion for 1 to 2 hours, subsequent to which the second phase of increased secretion continued for about 2 hours. The secretion curves following food for the two second stage dogs shown in figure 2 follow just such a course. Bickel and Wagner ascribe the first phase to reflexes initiated by the act of ingesting food and the second to a humoral-chemical mechanism and add the comment that in the dog it is uncommon for activity in one part of the intestinal tract to influence glandular activity in another part. The aid of this explanation of the mechanism cannot be invoked in the case of these animals because the loops were completely denervated, and in the absence of any evidence we are unable to offer any explanation of our own.

W. N. Boldyreff maintains that it is only the spontaneous "periodic secretion" which can be considered the natural secretion of the gut. An ex-

ception is made in the case of a particular type of mechanical stimulus described by him (1928). It is claimed further that the production of intestinal juice is not related to digestion and cannot be elicited by feeding. It is common knowledge that the diet of the average dog probably offers more mechanical stimulation to the intestine in the way of bones, cartilage, etc., than an ordinary smooth catheter and, therefore, it would appear to be rather difficult to define the "natural" secretion on such a basis. In regard to the relation of the production of intestinal juice to digestion, it is evident, from our quantitative enzyme studies as well as the volume produced, that feeding has a very decided effect—either inhibiting or accelerating the secretion, depending largely upon the integrity of the extrinsic nerves.

Discussion. The intestine, of course, carries on the processes of absorption and secretion simultaneously. To what extent, if any, reabsorption of secreted juice takes place under the conditions of collection employed here cannot easily be determined. Until it is ruled out, we must think of any increase in volume as affected to an unknown extent by possible reabsorption and that this process also may be influenced by food. In a closed loop the volume of fluid recovered certainly represents the sum of the two processes of reabsorption and secretion (Nasset and Parry, 1934). It is generally agreed that enzymes are either proteins or closely associated with proteins. The possibility of a change in the rate of reabsorption in response to a meal does not complicate the interpretation of the data on enzyme production, since the presence of an active proteinase in the intestinal juice is extremely doubtful. (See Pierce, Nasset and Murlin, 1935.) Therefore, as mentioned above, the enzyme studies are of utmost importance in the determination of the effect of a meal on intestinal secretion.

The point often has been raised that the enzymes of *succus entericus* are really intracellular enzymes liberated by cytolysis of desquamated epithelial cells. This can be shown to be highly improbable by a simple calculation. On many days, after a meal, it has been possible to collect in 6 to 8 hours 50 cc. of juice containing about 3 per cent of solids from a pair of loops each 10 cm. long and about 1.5 cm. in diameter. Were this amount of solids (1.5 gm.) incorporated into tissue containing 75 per cent water it would make a layer approximately 0.5 mm. in thickness over the whole inside area of the loops, i.e., the equivalent of the whole mucosal surface. Examination of the fresh juice has revealed very few epithelial cells and little debris indicative of cellular disintegration. However, several types of leucocytes were found but not in great numbers.[1]

[1] We are indebted to Dr. W. K. Smith of the Department of Anatomy for the microscopical examination of the specimens of intestinal juice.

The evidence presented shows clearly at least three components in the control of the secretion of isolated intestinal loops, namely: 1, local mechanical stimuli; 2, inhibitory influences exerted via the extrinsic nerves, and 3, the humoral stimulation brought about by digestion in another part of the GI tract. The first requires no comment since it was maintained constant throughout and is an observation which everyone who has worked with the intestine has made. The remaining two have been proposed before in various combinations but not unequivocally demonstrated because of the lack of assurance that every extrinsic nerve had been severed. It is believed that the method of transplantation with subsequent complete isolation from the mesentery obviates this objection.

The manner of release, in the normally innervated loop, of the inhibition initiated by food in the GI tract is as yet unknown. The conception of Savitsch (1922) that it is the local stimuli which accomplish this seems to be supported by considerable evidence. In several of the dogs (table 3) however, the combination of the presence of the catheter as the local stimulus with the humoral effect of a meal was insufficient to overcome the nervous inhibition and cause an increase in secretion. After complete denervation the same combination of stimuli produced copious secretion in the same dogs. It seems probable, therefore, that the local stimulus required may be the presence of food itself, i.e., a local mechanical and chemical stimulus in contact with the particular portion of the intestine concerned. In some dogs, on the other hand, the local stimulus afforded by the catheter apparently was sufficient and the response to a meal was essentially unaltered by complete denervation of the loop.

The demonstration of a humoral mechanism in the secretion of the jejunum harmonizes well with the knowledge concerning the control of gastric and pancreatic juice, and bile. There appears the added modification, discussed above, of a nervous mechanism which may inhibit secretion in those regions of the gut not receiving adequate local or humoral stimuli.

SUMMARY

Experimental evidence obtained from seven dogs demonstrates that:

1. In transplanted loops of jejunum with original innervation and circulation (1st stage), food in the digestive tract was accompanied by a decrease in rate of secretion in 3 dogs, and an increase in 4.

2. After complete isolation of the loop by division of the mesenteric pedicle (2nd stage), food in the digestive tract was accompanied by an increased rate of secretion in all dogs but one in which there was no great change. This proves the presence of a humoral control of the rate of secretion of fluid.

3. Enzyme production (sucrase, amylase-maltase, peptidase and lipase)

was decreased by food (1st stage) in 3 dogs and increased in 4. In the second stage preparations feeding augmented the production of all enzymes in all dogs excepting sucrase in 3, peptidase in 1, and lipase in 1, proving humoral control of the secretion of these organic constituents.

4. There is relatively little cellular material from the mucosa in intestinal juice as collected from these transplants. A calculation is made to show that it is highly improbable that the enzymes found arise chiefly from disintegration of desquamated cells.

5. The secretory activity of isolated loops is intermittent. The periods of greatest activity may be spaced 2 to 4 hours apart, which interval appears not to depend upon the integrity of the extrinsic nerves of the gut.

6. There are at least 3 components in the control of the secretion from isolated intestinal loops: *a*, local mechanical stimuli; *b*, inhibitory influences exerted via some of the extrinsic nerves, and *c*, humoral stimulation occasioned by digestion of food in another part of the GI tract.

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THERMAL SHORTENING OF NERVE

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Thermal shortening of nerve was described by Harless (1860), Brodie and Halliburton (1904) and Halliburton (1916). Brodie and Halliburton claimed that the shortening occurs in several steps and that the temperature at each step corresponds to that at which a definite nerve protein coagulates. Alcock (1903) had stated that irritability is abolished at a temperature corresponding to that at which the first of the body proteins coagulate and the conclusion was drawn by Alcock and by Brodie and Halliburton that thermal shortening and extinction of irritability are both manifestations of neuroprotein coagulation. These conclusions were criticized by Meigs (1909).

In this paper we attempt to localize the source of thermal shortening in nerve and to show that oriented protein primary valence chains of the axis cylinder are at least partially responsible for the phenomenon. Subsequently we shall describe experiments on the solvation and desolvation of nerve which confirm these conclusions.[1]

METHOD. To avoid tension and yet to record the shortening accurately we have used both an optical lever and a travelling microscope method. The optical lever system was similar to that described by Brodie and Hallburton (1904) except that the mercury was omitted and the sensitivity was enhanced by placing the mirror on a separate counterbalanced pulley. The apparatus is pictured in figure 1. With the second method the nerve is made to lie extended in a thin-walled glass tube, the inner bore of which is at least three times the diameter of the nerve. Being careful to fill the tube completely with solution in order to avoid air pockets it is then placed horizontally in a beaker of Ringer solution.[2] Length of the nerve is measured by a travelling microscope, temperature by a calibrated thermometer placed near the glass tube. The beaker is contained in a larger beaker of water, the temperature of which can be regulated suitably. It is of course essential that the nerve lie straight in the tube and that the heating be sufficiently slow so that the temperature as read on the thermometer ap-

[1] A brief summary of this work has already been presented (Schmitt and Wade, 1934).

[2] Petroleum oil was substituted for Ringer solution in some of the experiments.

proximates that on the inside of the tube. In control experiments it was found that rapid heating (3 degrees per min.) gives a curve similar to that obtained with slow heating (0.5 degree per min.) except that the initial portion of the curve is obscured and the temperature of rapid shortening is pushed in the direction of higher temperature (see table 1). The two methods give similar results when conditions are ideal. The optical lever, being more sensitive than the travelling microscope method, is more satisfactory for measurement of the small initial changes at temperatures below 46°. However, it is difficult to counterbalance the moving aluminum wire so that the nerve shall be just extended to its full length and yet not be under tension.

TABLE 1

Thermal shortening of peripheral nerve

NERVE	METHOD	RATE OF HEATING	SHORTENING RANGE	SHORTENING OF ORIGINAL LENGTH	WEIGHT		LIQUID MILIEU FOR TEST
					Before heating	After heating	
		°C. per min.	*°C.*	*per cent*	*mgm.*	*mgm.*	
Frog sciatic	Opt. lever	0.25	46–58	50	10.7	9.6	Oil
Frog sciatic	Opt. lever	0.91	48–70	45	18.6	14.1	Ringer
Frog sciatic	Trav. mic.	0.94	45–65	51	25.3	19.8	Oil
Frog sciatic	Opt. lever	1.31	49–66	52	24.6	20.8	Oil
Frog sciatic	Trav. mic.	3.40	49–65	56	24.9	19.1	Oil
Frog sciatic (split sheath)	Trav. mic.	0.85	46–60	65	5.0	4.4	Oil
Frog 9th motor root	Trav. mic.	0.90	45–61	43	8.1	8.4	Ringer
Limulus leg nerve	Trav. mic.	1.45	50–95+	28			Ringer
Limulus leg nerve	Trav. mic.	2.41	51–95+	28			Ringer
Lobster claw nerve	Opt. lever	0.86	50–70	66	58.0	19.6	Sea water
Lobster claw nerve	Trav. mic.	1.16	51–71	63			Sea water
Lobster claw nerve	Trav. mic.	0.72	52–67	58	91.2	30.5	Sea water

RESULTS. Heating at a steady rate of 0.5 to 1.0 degree per minute, there is a very small shortening beginning at about 36° and continuing until about 46° to 48°, the total shortening amounting to 1 to 2 per cent of the length of the nerve (see fig. 2). The curve of rapid shortening is smooth throughout and lies between 48° and 58° depending on the conditions and the type of nerve. Essentially the same type of curve was obtained with the travelling microscope method except that the period of rapid shortening occurred at a slightly lower temperature. Typical data for the shortening of various nerves are presented in table 1, the range of shortening being reckoned as that of the rapid shortening; the small preliminary shortening at the lower temperatures and the tailing out of the curve after the rapid shortening are not included. The curves show little

resemblance to those of Brodie and Halliburton. There is a general similarity in that shortening begins very slowly at 36° and proceeds rapidly somewhere in the neighborhood of 50° to 52°, but the definite inflections shown in their curves were absent in ours (except for the inflection at the end of the initial slow period). Since Brodie and Halliburton consider the inflections evidence of the presence of four distinct neuroproteins we have examined this point in some detail. The curves in figure 2 and the data in table 1 were obtained by heating the nerves at a steady rate. Brodie and Halliburton, however, heated irregularly; when considerable shortening occurred, the temperature was held constant until the shortening stopped. To reproduce such conditions we constructed an electrical heating device by means of which the temperature could be increased at any desired rate or held constant at any time. With this apparatus, proceeding in the manner of Brodie and Halliburton, we found that we could obtain inflections in the curve at will, depending on how the heating was manipulated. That these inflections are probably meaningless is shown by the following experiment. Nerves were suspended unweighted, each in a separate beaker of mineral oil and kept at a definite constant temperature from the moment of immersion. From time to time each was withdrawn, hung vertically, and the length measured by means of a travelling microscope. After it became apparent that there was no longer any shortening the temperature was rapidly raised to 70° to determine the relative completeness of shortening at the lower temperature. The results are shown in figure 3. For the first hour or so after nerves are dissected they may shorten 2 to 3 per cent even at 25°. At 35° to 44° the shortening may amount to 6 to 10 per cent of the length of the nerve[3] and at 46° the maximum shortening may be 21 per cent. Above 48° the shortening will proceed slowly until the nerve has been maximally shortened. It appears therefore that there is a critical point in the neighborhood of about 48° at which the protein chains shorten maximally. Ewald (1919) and Wöhlisch (1926) obtained similar results for tendon. The step-like curves of Brodie and Halliburton, therefore, cannot be regarded as evidence of the existence in nerve of the several proteins which are coagulated in brain extracts at similar temperatures. This conclusion is borne out by the chemical investigations of McGregor (1917) and Block and Brand (1933).

Structural elements responsible for thermal shortening. The structural units in peripheral nerve may be classified under three general categories: myelin sheath, connective tissue and other sheath material, and axis

[3] In St. Louis the room temperature during the summer months may regularly be 32 to 35°C. These experiments show the necessity of keeping the temperature of nerve below 25° from the moment of dissection.

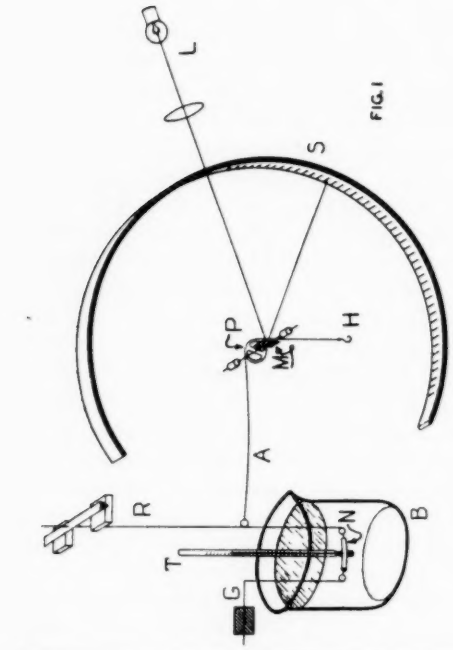

FIG. 1

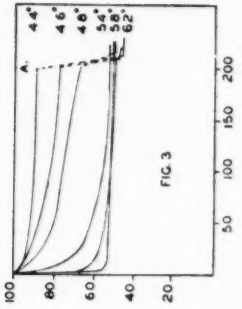

FIG. 3

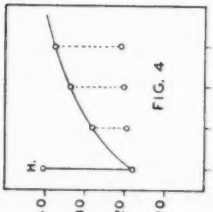

FIG. 4

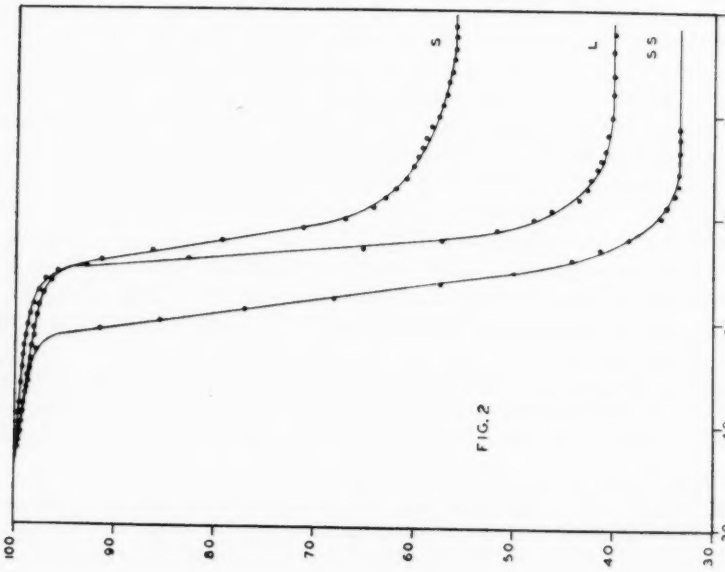

FIG. 2

cylinder. Of these we may eliminate the myelin sheath as a significant source of thermal shortening, for as shown in table 1, there is no correlation between the presence of myelin and the ability to shorten. Moreover long extraction of frog sciatics with alcohol has little effect on the total shortening or on the temperature range of shortening although under these conditions the myelin is almost completely removed. That the fibrous connective tissue of frog sciatic nerve is capable of thermal shortening much the same as other connective tissue we were able to demonstrate directly. In frog sciatics soaked in N/100 HCl, the interfibrillary connective tissue becomes greatly swollen and it is easy to separate long strips of connective tissue from the axons by teasing under the dissecting microscope. Heating these strips results in vigorous shortening, the temperature of shortening being rather indeterminate because of the acid swelling. Dissecting out long stretches of axon from the same preparation, we were able to demonstrate that single axons freed of connective tissue also show thermal shortening strikingly, becoming greatly rolled up and convoluted. While interfibrillary connective tissue undoubtedly plays a rôle in thermal shortening the phenomenon must be due in the main to the axons themselves for spinal roots and lobster claw nerves which contain little or no

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Fig. 1. Optical lever system for determining thermal shortening of nerve. A piece of aluminum wire, *R*, two feet in length is suspended from a "frictionless" bearing, so that its lower end dips below the surface of the liquid in a large beaker. One end of the nerve, *N*, is fastened to the free end of the wire by means of fine silk thread; the other end of the nerve is tied in a similar manner to a stationary vertical rod, *G*, which also dips below the surface of the liquid. Movement of the nerve is amplified optically in the following manner. A fine silk thread, *A*, leads from the aluminum wire over a light pulley system, *P*, the tension on the thread, and hence on the nerve, being regulated by minute weights on hook *H*. Movement of the pulley is recorded by reflection of a spot of light onto the circular scale *S*, from the mirror, *M*, mounted on the shaft of the pulley. Under the usual operating conditions displacement of the tip of the aluminum lever is amplified about 50 times by this system

Fig. 2. Thermal shortening curves. Ordinates represent length of nerve in per cent of original length at room temperature; abscissae represent temperature in degrees, Centigrade. Curve *S* is for frog sciatic nerve in Ringer solution (optical lever). Curve *S S* is for frog sciatic nerve with split sheath, measured in mineral oil (travelling microscope). Curve *L* is for lobster claw nerve measured in sea water (optical lever).

Fig. 3. Effect of prolonged heating at constant temperatures on length of nerve. Ordinates as before; abscissae, time in minutes after immersion of nerves. At *A* the temperature of each preparation was rapidly raised to 70° to determine the maximal shortening.

Fig. 4. Elasticity of heated nerve. Ordinates represent length of nerve as read by travelling microscope; abscissae represent weight in grams, hung onto the nerve to extend it. *H* represents the original length before heating. Dotted lines show reversibility of extension determined by removal of weight.

connective tissue show thermal shortening even more strikingly than frog sciatics. While the above appears to rule out interfibrillary connective tissue as the major source of shortening, it is difficult to exclude the Schwann sheath of medullated nerves by direct evidence. The physical and chemical nature of this sheath is poorly understood (see Maximow and Bloom, 1930; Ettisch and Jochims, 1927; de Renyi, 1928) but if it is constructed of typical connective tissue, it would be a possible source of thermal shortening in the individual axon. However, the microscopic study about to be described provides evidence that the axis material actively shortens when heated and the general picture can be interpreted best on the assumption that the Schwann sheath plays at best a minor rôle.

For the microscopic examination a frog sciatic was cut in two and the proximal portion heated to 60°C., the distal portion being kept at room temperature as a control. Both halves were then fixed in osmic acid, imbedded and sectioned. Sections from control and heated nerve were obtained from closely adjacent regions, hence the difference in appearance is due to the shortening process. Figure 5, A-D are microphotographs of these sections. Perhaps the most striking change produced by the heating is the increase in the cross sectional area of the entire nerve and the marked increase in the cross sectional area of the individual axons at the expense of the interfibrillary spaces and connective tissue. Longitudinal sections are even more striking. Figure 6, A-D, are microphotographs of sections made from material prepared as follows. The left eighth motor root of a bullfrog was placed in Ringer solution at 65°C. for 30 seconds. Some minutes later it was placed in osmic acid along with the unheated eighth motor root which was kept as a control. Both nerves were imbedded and sectioned longitudinally. Again the increase in thickness of the nerve as a whole and of the individual axis cylinders is evident. In figure 6, D, the neurofibrils in several fibers are shown to be clumped in the center of the axis in a manner such as to leave no doubt of their inherent contractility or of the fact that their shortening produced telescoping of the fiber. In teased fibers of nerves heated to 53 or 55° Engelmann (1880) observed that the neurofibrillar strands retracted from the nodes several hundredths of a millimeter with accompanying shortening of the internodal region of the fiber.

In order to observe the shortening process in single teased fibers devoid of sheath restraints we constructed a cell which permitted the temperature to be controlled by means of water perfusion and made of glass so that the fibers could be examined with the high dry or oil immersion lens. As the temperature is raised there is first a tendency for the myelin to buckle at various points and to produce isolated droplets in the axis. Simultaneous with this is the appearance of fibrils. Finally, after a very brief period in which the fiber begins to move slightly in the solution and to give indica-

tion of shortening, it undergoes very rapid shortening, becoming twisted and rolled upon itself. This shortening of single fibers is not reversed by cooling. In a few instances axis material was observed to move violently through a portion of an internodal segment at the moment of rapid shortening. This was interpreted as direct evidence of the contractility of the

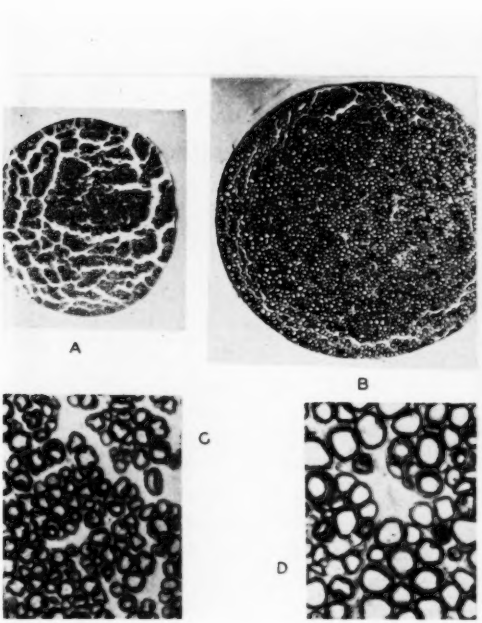

Fig. 5

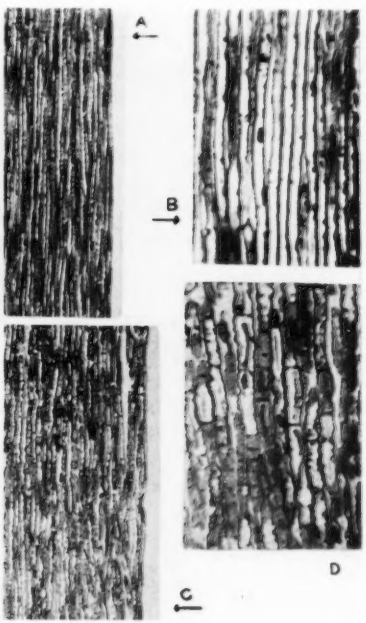

Fig. 6

Fig. 5. Effect of thermal shortening on nerve structure. *A* and *B* are cross sections of control and heated portions, respectively, of a frog sciatic nerve (magnification = 72×). *C* and *D* are enlargements of *A* and *B*, respectively (magnification = 425×). Further description in text.

Fig. 6. Effect of thermal shortening on nerve structure. *A* and *C* are longitudinal sections of control and heated portions, respectively, of eighth motor root of bullfrog (magnification = 70×). *B* and *D* are enlargements of *A* and *C*, respectively (magnification = 200×). Further description in text.

axis, although it is difficult to be sure that the effect is not due to propulsion of axis material by contraction of portions of the fiber immediately adjacent. Since the myelin is organically connected with the axis (de-Renyi, 1928) it is to be expected that it would be thrown into folds by the shortening. This folding in of the myelin would be increased at the mo-

ment of rapid telescoping of the fiber, and since the nodes are regions of relative solidity as compared with the internodes, one would expect the internodal regions to be more bulged by the contracture than the nodes. This may be verified from figure 6-*D*.

Reversibility of thermal shortening of nerve. The degree of reversibility of shortening depends to a certain extent upon the duration of heating. As shown in table 2, if a nerve is immersed very briefly in hot Ringer solution, the small amount of shortening may be reversed almost completely, and such brief heating, moreover, is associated with no loss in weight. Splitting the epineural sheath further increases the regain somewhat. Long drawn out heating is associated with distinctly less tendency for subsequent elongation. Soaking the nerves in formalin does not greatly affect the immediate regain in length although the final regain is usually higher, amounting to 40 or 50 per cent in some instances. Essentially similar results were obtained with bullfrog motor roots. Reversi-

TABLE 2

Reversibility of shortening of frog sciatic nerve

LENGTH BEFORE HEATING	TIME IN RINGER SOLUTION AT 70°C.	LENGTH AFTER HEATING	LENGTH IN RINGER SOLUTION AT 20°.C					SHORTENING OF ORIGINAL LENGTH	MAXIMUM REGAIN OF SHORTENING
			After 1 min.	After 15 min.	After 7 hrs.	After 24 hrs.	After 48 hrs.		
mm.	*sec.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*per cent*	*per cent*
44.5	0.5	38.0	41.0	41.0	42.5	42.5	43.5	15	85
44.0	3	28.0	29.0	30.0	33.0	34.5	34.0	36	41
45.0	15	24.5	25.5	25.5	28.0	28.5	29.0	46	29
45.0	120	23.5	24.5	24.5	26.5	26.0	26.0	48	14

bility of shortening of nerve apparently depends on the degree to which thermal desolvation of the primary valence chains has been pushed. In confirmation of Peterfi (1929), we find that warming nerve to 35 or 37° greatly accelerates the formation of neurofibrils. It would appear, therefore, that the first result of heating is aggregation of the preëxisting highly solvated primary valence chains, through secondary valence lateral adhesions, forming visible fibrils. If the nerve is heated further, the primary valence chains buckle due to removal of structure water, and shortening occurs. The limited reversibility even with formolized nerves is probably due to the relatively imperfect orientation and high degree of solvation of nerve as compared with tendon or hair.[4]

Elasticity of heated nerve. A frog sciatic dipped in Ringer solution or in oil at 60 to 70° for a few seconds shortens about 50 per cent and obtains a

[4] For a discussion of the theoretical significance of the reversibility of thermal shortening of oriented primary valence chains see Wöhlisch and du Mesnil de Rochemont (1927), Meyer (1929) and Küntzel and Prakke (1933).

rubber-like elasticity. Such a nerve may be stretched almost to its original length without rupture and the process is reversible (see fig. 4). This elasticity may be demonstrated best with split-sheath preparations or with individual fibers and is due in the main, therefore, to the axons rather than to interfibrillary connective tissue. It is exhibited, moreover, by heated motor roots and by claw nerves of the lobster quite as strikingly as by frog sciatics and hence must be attributed in great measure to the primary valence chains of the axis proteins, although connective tissue, which is present so abundantly in a nerve such as the frog sciatic is also elastic when heated and must contribute to the phenomenon in the case of this nerve. In analogy to the theory of Meyer (1929) and Küntzel and Prakke (1933) and supported by x-ray diffraction studies (unpublished) it is to be supposed that heat-shortened nerve is elastic because it is possible by stretching to cause the primary valence chains which were greatly buckled by heating to become partially extended and oriented once more as in the unheated tissue.

SUMMARY

1. Peripheral nerve shortens when heated, the amount of shortening and the temperature range depending on the type of nerve and the experimental conditions. Frog sciatics and motor roots shorten 40 to 50 per cent, the range for moderately fast heating being 48 to 60°C. Splitting the epineural sheath increases the shortening and lowers the range. Leg nerves of Limulus shorten 30 per cent in the range of 54 to 90°; claw nerves of the lobster shorten 60 to 70 per cent in the range of 52 to 85°.

2. The inflections in the shortening curve which Brodie and Halliburton claimed represent the coagulation points of the various neuroproteins are probably artifacts. The question of the critical nature of the shortening temperature and the effect of the rate of heating are discussed.

3. Connective tissue in nerve shows thermal shortening and must be responsible for a portion of the total shortening. But in bullfrog motor roots where there is little connective tissue, the shortening is due chiefly to the axons themselves and cytological evidence suggests that materials in the axis cylinder are responsible for the phenomenon.

4. Heat-shortened nerves tend to elongate somewhat when cooled; they show striking elasticity and can be stretched fairly reversibly to their original length without rupture; tension prevents shortening but if a nerve has been heated under tension, cooled and then reheated, it commences to shorten as low as 26° and shows no indication of rapid shortening at a critical temperature.

5. These facts place nerve in the same category as the collagen fibrils of tendon and skin and indicate the presence of oriented protein primary valence chains in the axis cylinder.

We wish to acknowledge the assistance of Mr. Henry Breymann in the experimental work reported in this paper.

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SOLVATION AND DESOLVATION OF NERVE

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In the preceding paper (Schmitt and Wade, 1934b), it was shown that thermal shortening of nerve can be interpreted as being due, at least partially, to the buckling of protein primary valence chains of the axis cylinder, caused, perhaps, by increased agitation of the chains and by desolvation. It was also stated that these primary valence chains in nerve behave towards solvating and desolvating agents in a manner similar to those of typically fibrous tissues such as tendon (Schmitt and Wade, 1934a). In the present paper we furnish details of the experiments on solvation and desolvation.

Solvation. Protein fibers when subjected to the action of agents which solvate the polar groups, usually swell, the fibers shortening and thickening and in some cases increasing considerably in weight. While water is itself a good solvating agent, certain other substances of high dipole moment are far superior; of these formamide, thiocyanate, acids, bases, etc., are preëminent. These substances have long been known to produce swelling shortening of tendon and muscle but little information is available concerning their action on nerve.[1] Bauer (1912) claimed that dilute acid had a desolvating action on the proteins of mammalian spinal cord. This view was based on the fact that discs of cord material gained more weight in distilled water than in dilute acid; no longitudinal shortening was mentioned. Spiegel (1921) observed changes in double refringency under the influence of acids and explained the effect entirely on the swelling of the myelin sheath due to changes in the amount and position of water in the fluid crystals of the myelin. Ettisch and Jochims (1926), studying the effect of various reagents on the ultramicroscopic structure of teased fibers of frog sciatics, found nerve very sensitive to changes in pH. The effects were attributed largely to the swelling of the neurilemma and axiolemma sheaths which these authors regard as typical connective tissue. These results were not confirmed by Auerbach (1929).

Data from typical experiments are presented in tables 1 and 2. Summarizing the results it may be said that all of the solvating reagents tried produced results on medullated and non-medullated nerve comparable in

[1] The original papers of Vassil'ev (1922) and of Goldenberg (1929) were unfortunately not available.

most respects with those produced by the same reagents on tendon fibers. There is an initial shortening amounting to 10 to 60 per cent of the original length of the nerve, depending upon the reagent, the type of nerve, and the temperature. Thiocyanate and dilute acid produce shortening most strikingly. At a temperature of 30°C. the nerves shorten almost instantly they are dipped in the solution. Whether or not the solvated nerves show

TABLE 1

The effect of solvating agents on medullated nerve

TISSUE	REAGENT	CONC.	TIME IN SOLUTION	TEMP. OF SOLUTION	SHORTENING			CHANGE IN WEIGHT	
					After soaking	After heating	Range	After soaking	After heating
		M.	*hrs.*	*°C.*	*per cent*	*per cent*	*°C.*	*per cent*	*per cent*
Frog sciatic.....	Ringer		23	12	2	50	48– 60		
Frog sciatic.....	$HCONH_2$	0.25	21	12	18	34	48– 76	+34	−14
Frog sciatic.....	$HCONH_2$	2.0	21	12	14	46	28– 70	+7	−28
Frog sciatic.....	$HCONH_2$	14.0	144	12	23	54	30–110	+17	
8th sensory root...	$HCONH_2$	2.0	16	12	20	47	37– 60	+27	+8
Frog sciatic.....	NaCNS	0.12	22	12	−9	40	47– 63	+14	−4
Frog sciatic.....	NaCNS	2.0	23	12	58	59	None	−14	−18
Frog sciatic.....	NaCNS	2.0	7 min.	35			None		
9th sensory root...	NaCNS	2.0	20	12	55			+11	
Frog sciatic.....	HCl	0.1	39	12	24	64	24– 75	+33	−23
Frog sciatic.....	HCl	0.01	40	12	45	68	27– 85	+283	+52
Frog sciatic.....	HCl	0.001	41	12	5	44	49– 78	+61	−9
Frog sciatic.....	HCl	0.001	112	12	41			+247	
Frog sciatic.....	HCl	0.0001	16	12	6			+113	
8th motor root....	HCl	0.01	20	12	43			+283	
8th motor root....	HCl*	0.01	46	12	30			+63	
Frog sciatic.....	NaOH	1.0	20	12	35		31– 45	+44	
Frog sciatic.....	NaOH	0.1	22	12	15	56	37– 62	+49	−14
Frog sciatic.....	NaOH	0.01	15	12	14	65	36– 62	+48	−35
Frog sciatic.....	NaOH	0.001	17	12	2	50	26– 63	+108	+2
Frog sciatic.....	NaOH	0.0001	19	12	3	52	27– 60	+126	−17
Frog sciatic.....	H_2O		19	12	2	51	42– 78	+63	−30
Frog sciatic.....	H_2O		16	12	6			+163	

* HCl is made in Ringer solution.

considerable swelling shortening, the thermal shortening curve is very different from that of untreated nerves. Instead of shortening in a fairly well defined range somewhere between 46 to 60°C., they commence shortening as low as 28 to 30° and continue along a rather smooth curve showing no abrupt transition (see figs. 1 and 2). Küntzel and Prakke (1933) found that the lowering of the shortening temperature of tendon was an

inverse function, within limits, of the concentration of the acid. Swelling shortening on the other hand was maximum in the concentration range between M/100 and M/1000 HCl. Essentially similar results were obtained for nerve (see tables 1 and 2); maximum shortening and increase in weight at concentrations between M/100 and M/1000 HCl. Although swelling shortening in M/100 HCl is striking in medullated nerve, it is very limited in non-medullated nerve, although the temperature range of thermal shortening is considerably lowered in the latter case. It was desirable therefore to determine what structures in medullated nerve are responsible for swelling in acid. For this purpose we soaked frog sciatics

TABLE 2

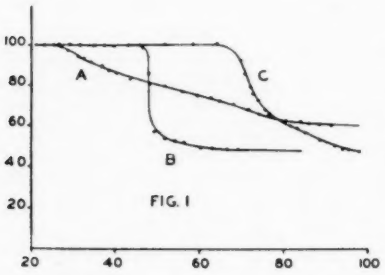
The effect of solvating agents on non-medullated nerve

TISSUE	REAGENT	CONC.	TIME IN SOLUTION	TEMP. OF SOLUTION	SHORTENING			CHANGE IN WEIGHT	
					After soaking	After heating	Range	After soaking	After heating
		M.	*hrs.*	°*C.*	*per cent*	*per cent*	°*C.*	*per cent*	*per cent*
Lobster claw nerve.	$HCONH_2$	2.0	16	12	14	65	40–62	+5	−77
Lobster claw nerve.	$HCONH_2$	2.0	15	30	29	63	46–60	−12	−74
Lobster claw nerve.	$HCONH_2$	7.0	18	12	17	78	28–80		
Limulus leg nerve..	$HCONH_2$	14.0	21	12	16	48	35–86	+20	
Lobster claw nerve.	NaCNS	0.12	14	12	4			+78	
Lobster claw nerve.	NaCNS	2.0	14	12	57			+33	
Lobster claw nerve.	NaCNS	2.0	2 min.	35	66		None		
Lobster claw nerve.	HCl	0.1	22	12	23		48–66	−12	
Lobster claw nerve.	HCl	0.01	17	12	20	63	28–70	+38	−77
Lobster claw nerve.	HCl	0.001	15	21	16			+39	
Limulus leg nerve..	HCl	0.12	45	12	0		28–65		
Limulus leg nerve..	NaOH	0.12	20	11	0			+104	−72
Lobster claw nerve.	NaOH	0.01	17	11	10	65	28–75	+210	−65
Lobster claw nerve.	H_2O		17	11	11	73	30?–65	+70	−78

and bullfrog motor roots in M/100 HCl for 24 hours and, after washing out the superficial HCl, fixed them in osmic acid and prepared cross and longitudinal sections. Figure 3 shows microphotographs of these sections. Striking swelling occurred in the Schwann sheath which took on an extremely tortuous appearance. In the loose folds of this swollen sheath may be seen a substance which darkens with osmic acid and which must correspond to myelin. However, to what extent the myelin was also swollen it is difficult to say from these sections. From longitudinal sections it is evident that the neurofibrillar material which is clumped into long strands was also considerably swollen. It would appear from these preparations that while Spiegel may be correct in stating that the myelin

sheath swells in dilute acid, the Schwann sheath also swells and reacts in this regard like typical connective tissue as claimed by Ettisch and Jochims.

In the absence of reliable data on the isoelectric point of nerve proteins, these swelling effects in dilute acid appear to be explained best by assuming that the binding of H ions results in an unequal distribution of ions within the structure of the micelle. This in turn sets up a type of membrane equilibrium which results in the entrance of water into the micelle according to the classical theory of Procter and Wilson (1916). And since the primary valence chains are bound together at intervals this water produces lateral bulging which results in shortening of the fiber. Although this swelling occurs both in the interfibrillary connective tissue and in the



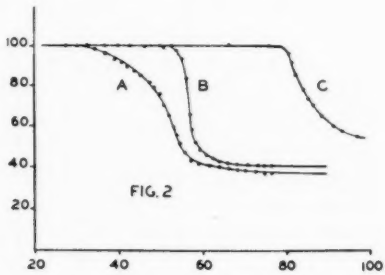


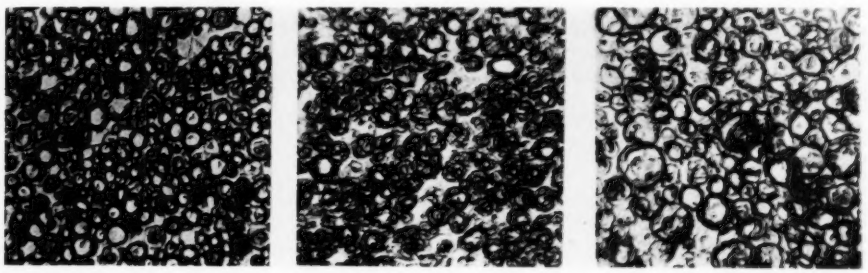
Fig. 1. Effect of reagents on thermal shortening of frog sciatic nerve. Curve *A* is for a nerve which had soaked for 40 hours in M/100 HCl; *B*, after 195 hours in 95 per cent alcohol at 35°C.; *C*, after 2.7 hours in 0.4 per cent HCHO. Ordinates represent length of nerve in per cent of that immediately before heating, abscissae, temperature in degrees Centigrade.

Fig. 2. Effect of reagents on thermal shortening of lobster claw nerve. Curve *A* is for a nerve which had soaked for 17 hours in M/100 HCl; *B*, after 40 hours in toluol; *C*, after 1 hour in 40 per cent HCHO. Coördinates as before.

Schwann sheath, it occurs also in lobster claw nerve containing predominantly axis material. The assumption seems justified therefore that the axis in medullated nerve contributes in part to the phenomenon.

Meyer (1929) found that tendon contracted by formamide has a rubber-like elasticity, due to the fact that the solvated primary valence chains which have become buckled and coiled upon themselves may be stretched out by tension. We have observed similar effects in the case of medullated and non-medullated nerve treated with formamide or with thiocyanate. After shortening over 50 per cent in the reagent, nerve so treated may be extended reversibly to and beyond its original length without rupture. This elasticity is particularly striking in the case of lobster nerve treated with thiocyanate.

Desolvation. Desolvation of protein micelles may be accomplished either by transformation of the proteins into a water-poor modification by heat or by dehydration either by increase of osmotic pressure or by organic dehydrating media. The first of these was described in the previous paper (Schmitt and Wade, 1934b). Regarding the effect of dehydration, it may be said that as in the case of other fibrous tissues, the shortening temperature of nerve depends largely upon the water content. Dehydration by acetone, toluol, and ether produces considerable shortening even at 12° and in some cases (e.g., acetone) increases the shortening temperature greatly (see table 3). Raising the osmotic pressure produces relatively little shortening or increase of shortening temperature. It appears that the intramicellar structure water cannot be removed thus easily. Indeed in relatively concentrated solutions of NaCl both medullated and non-medullated nerves increase in weight and shorten at rather low tempera-



A *B* *C*

Fig. 3. Effect of solvating agents on structure of frog sciatic nerve. *A*, is a section of untreated nerve as a control; *B*, section of a nerve after soaking for 15 hours in M/100 HCl; *C*, section of a nerve after soaking for 15 hours in 3M formamide in Ringer solution. Magnification = 330 ×. Further details in text.

tures as though the salt were acting as a swelling agent, solvating the polar groups rather than removing water.

Intermicellar chemical bridges. Ewald (1890, 1919) discovered that if connective tissue or tendon be given a preliminary treatment with formalin, potassium dichromate, or osmic acid, the minimum temperature at which the tissue shortens is considerably raised. Whereas certain untreated preparations shortened in the range 63 to 65°, after treatment with osmic acid comparable tissues shortened in the range 78 to 84°. This phenomenon has been interpreted in terms of the micellar theory by Meyer (1929) in the following manner: the formalin unites the primary valence chains laterally by the establishment of CH_2 bridges (Vernäheffekt[2]) integrating

[2] It may be pointed out that in the "Vernäheffekt" is to be seen the probable explanation for the widespread use of formalin, osmic acid, chromates, etc., in cytological fixatives. These substances, by unification of micellar structure through

TABLE 3

The effect of desolvating agents on nerve

TISSUE	REAGENT	CONC.	TIME IN SOLUTION	TEMP. OF SOLUTION	SHORTENING			CHANGE IN WEIGHT	
					After soaking	After heating	Range	After soaking	After heating
		per cent	*hrs.*	°C.	*per cent*	*per cent*	°C.	*per cent*	*per cent*
Frog sciatic nerve	Acetone	100	20	12	17	52			
Frog sciatic nerve	Toluol*	100	71	35	20			−83	
Lobster claw nerve	Toluol	100	40	15	18	68		−44	−84
Frog sciatic nerve	Alcohol	95	195	35	8	54	45–60	−43	−66
Frog sciatic nerve	Alcohol	95	432	12	0	36	50–63		
Frog sciatic nerve	Alcohol-ether (1:1)	95	195	35	5	47		−70	−67
Frog sciatic nerve	$(NH_4)_2SO_4$	Sat.	15	12	22	50	70–105	−51	−62
Frog sciatic nerve	NaCl	16	16	12	8			+21	
Frog sciatic nerve	NaCl	11.6	22	12	8	30	53–75	−10	−6
Frog sciatic nerve	NaCl	5.8	19	12	8	48		+25	+8
Lobster claw nerve	NaCl	11.6	17	12	9	70	41–70	+31	−68

* Tube containing nerve shaken continuously to ensure extraction.

TABLE 4

The effect of formaldehyde on the thermal shortening of nerve

TISSUE	REAGENT	CONC.	TIME IN SOLUTION	TEMP. OF SOLUTION	SHORTENING			CHANGE IN WEIGHT	
					After soaking	After heating	Range	After soaking	After heating
		per cent	*hrs.*	°C.	*per cent*	*per cent*	°C.	*per cent*	*per cent*
Frog sciatic nerve	HCHO	4.0	4.5	12	2	45	81–95	0	−52
Frog sciatic nerve	HCHO	0.4	2.7	12		52	69–96		
Frog sciatic nerve	HCHO	0.04	22	12		50	66–90		
Frog sciatic nerve	HCHO	0.01	18	12	6	34	53–85	−5	−25
8th sensory root	HCHO	0.4	18	12	13	58		+113	−24
Limulus leg nerve	HCHO	20.0	22	12	13	53	61–100	+6	
Lobster claw nerve	HCHO	40.0	1	12		50	73–105		
Lobster claw nerve	HCHO	10.0	19	12	13	70	70–110		
Frog sciatic nerve	Na_2CrO_4	2.85	19	12	19	38		+1	−36
Frog sciatic nerve	OsO_4	1.0	40	12	4	18		+20	−2
Lobster claw nerve	OsO_4	1.0	40	12	8	46		−5	−55

the structure of the fiber and making it necessary to supply more thermal energy to produce buckling of the primary valence chains in thermal

the formation of intermicellar chemical bridges raise the shrinkage temperature to such a point that the process of imbedding the tissue (oven temperature is usually about 62°C.) does not produce thermal shrinkage.

shortening than in the case of the untreated fiber. Since this reaction appears to be a test for the presence of longitudinally oriented protein primary valence chains we have applied it to nerve. It will be seen from table 4 and figures 1 and 2, that these reagents produce a striking increase in the shortening temperature of each type of nerve tested. The degree of temperature elevation depends on the concentration of the reagent and on the duration of the treatment. As with the solvating and desolvating agents, there can be no question but that formalin, osmic acid, etc., exert an effect on the connective tissue sheaths and perhaps also on the Schwann sheath in medullated nerve as well as on the axis cylinder. However there is no correlation between the degree of the effect and the quantity of connective tissue; lobster claw nerves and motor roots show the effect equally as strikingly as frog sciatics. The conclusion follows, therefore, that the effect may be traced in part at least to the axis cylinder and as such may be considered supporting evidence for the existence within the axis cylinder of longitudinally oriented primary valence chains.

SUMMARY

1. Solvating agents such as formamide, thiocyanate, acids and alkalies produce considerable shortening and increase in weight of nerve even at room temperatures and the temperature of thermal shortening is greatly lowered. Desolvation as by the action of toluol, ether, acetone, etc., may produce shortening and increase in the temperature range of thermal shortening. Unification of the micellar structure by "tanning" with formaldehyde, osmic acid, etc., greatly increases the temperature of thermal shortening.

2. Experiments with each reagent have been done on frog sciatics, bullfrog motor roots and claw nerve of the lobster. By comparing the relative amount of axis, connective tissue and myelin in these types it is possible to conclude that the above reactions are produced largely by the axis cylinder, although connective tissue when present behaves similarly. There is evidence that the Schwann sheath swells in dilute acid and in formamide.

3. These results are considered contributory evidence of the existence in the axis cylinder of long protein primary valence chains oriented parallel with the long axis of the fiber.

We wish to acknowledge the assistance of Mr. Henry Breymann in the experimental work reported in this paper.

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THE MONOPHASIC ELECTROGRAM OBTAINED FROM THE MAMMALIAN HEART

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In the development of an account of the T wave in the electrocardiogram, use has been made of variation in contour, duration and phase relationship of the monophasic electrocardiogram of various portions of the heart (Katz and Weinman, 1; Katz, 2). Such variations have been demonstrated to occur in the cold blooded animal (Yoshida, 3) but no equivalent observations have been made in the mammal because of the technical difficulties in obtaining monophasic electrocardiograms. The problem has been further complicated by the recent assertion of Wilson et al. (4) that the monophasic electrogram is a register of the potential variations in the injured region and not, as has been generally accepted, in the uninjured region upon which the second recording electrode is placed. It became important, therefore, to develop a method of obtaining the monophasic electrogram in the mammal, in order to test whether it is a record of the injured or uninjured regions.

METHOD EVOLVED FOR PRODUCING PERSISTENT MONOPHASIC CURVES, WITH NOTES ON THE SPECIAL ELECTRODE USED. Monophasic curves are easily obtained in direct leads from the cold-blooded heart by burning or chemically injuring the tissue beneath one lead-off electrode, but the intrinsically rapid recovery of the warm-blooded heart muscle makes it impossible to record comparable monophasic currents from the mammalian heart by this method. If a spot on a dog's heart[2] is so injured, the region quickly becomes functionally "walled off' from the uninjured regions, thus becoming merely a mass of dead tissue acting as a part of the lead-off electrode attached to it. A monophasic response may be obtained in this way, but it is extremely transitory, becoming diphasic within a few seconds or minutes after the injury is produced.

Better results were obtained by burning a small hole completely through

[1] Aided by the Frederick K. Babson Fund for Study of Diseases of the Heart and Circulation, Michael Reese Hospital.

[2] Dogs anesthetized with morphine and barbital were used, the chest being open and respiration maintained artificially.

the ventricular wall with a red hot needle 1.5 mm. in diameter, and inserting the wool wick of one electrode into the hole, but here too the monophasic character of the electrical response was not persistent enough to allow observations to be made over a sufficiently long period of time. The problem, then, was to find a method of injuring a small portion of tissue sufficiently to cause it to maintain its injured state but not enough to destroy it completely. Schütz (5) has described a method of producing monophasic electrocardiograms from the mammalian heart by leading off from an injured area produced in a small mass of muscle by sucking it up with a vacuum pump and then ligating it. After trying this and several other methods, we have selected a method in this laboratory in which the injury produced is minute both in extent and degree, but is still sufficient to yield satisfactory and persistent monophasic curves.

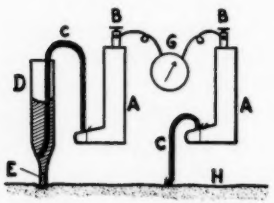

Fig. 1. Arrangement for recording monophasic electrograms. *A*, nonpolarizable boot electrode; *B*, zinc strips; *C*, woolen yarn wicks; *D*, glass electrode; *E*, tip of glass electrode plugged with moist cotton; *G*, string galvanometer; *H*, heart surface.

For this purpose a special electrode was developed (fig. 1 *D*) to produce the injury and at the same time act as a lead-off electrode from the injured spot. This electrode consists of a glass tube 8 cm. long and 6 mm. in diameter, drawn out at one end to a capillary tube 1.0 or 1.5 mm. in diameter. The bore of the capillary is plugged with moist cotton, *E*, and the larger end of the tube is partly filled with 0.9 per cent saline into which the woolen yarn wick from the tip of the usual non-polarizable boot electrode, *A*, is allowed to dip. The lead off electrode from the uninjured spot consists of a similar boot electrode, the woolen yarn wick from which is fastened to the surface of the heart. Great care is employed to avoid creating any injury at this point by using a very fine suture and passing it through the epicardium only. It was found advantageous to keep the moisture content of the wick, *C*, constant by impregnating the yarn with 0.3 per cent agar–0.9 per cent saline solution. Both boot electrodes are connected to an ordinary string galvanometer, *G*, which is made sufficiently sensitive to obtain a curve about 1 cm. in amplitude.

In producing the injury, moderate pressure is necessary in applying the tip of the glass electrode to the heart. Light pressure on the one hand, by avoiding injury, and heavy pressure on the other, by completely destroying the tissue are unsuitable and both give unsatisfactory curves. With little experience the proper pressure can be found which gives rise to a monophasic response and this response will persist unchanged in contour and duration as long as the pressure is maintained. We have attempted to

use mechanical devices to produce this moderate compression, but as yet have not been able to get as good results as with manual compression. The small area of muscle beneath the electrode appears blanched and cyanotic but when the pressure is lessened it regains its normal color within a minute or two and diphasic electrical complexes are again recorded.

PRELIMINARY EXPERIMENTS TO CHECK THE METHOD OF USING THE ELECTRODE. Figure 2 illustrates an experiment typical of several performed in which the direction of the monophasic curve was reversed by using two special glass electrodes to lead off the current, and compressing each in turn. Curve A is the monophasic curve obtained by applying electrode *1* to the heart

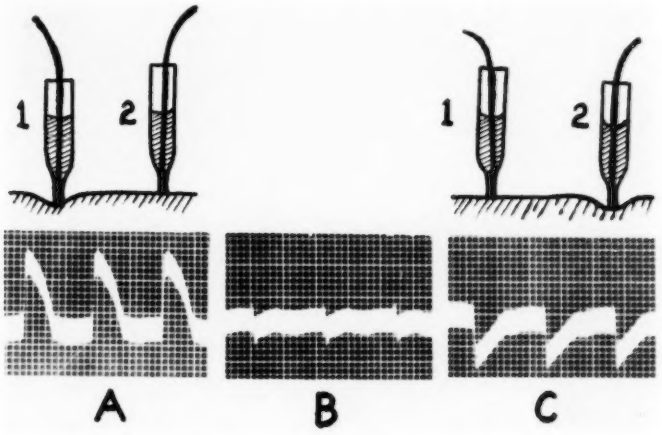

Fig. 2. Curves to demonstrate reversible character of injury currents with technique employed. A, monophasic electrogram obtained with moderate pressure on electrode *1*, light pressure on electrode *2*; B, curve obtained 26 seconds after releasing pressure on electrode *1* and increasing pressure on electrode *2*; C, monophasic curve obtained 3 seconds after B.

with moderate pressure and electrode *2* with very light pressure; it is a typical response obtained from an injured-uninjured pair of spots. Pressure on electrode *1* was then released and pressure on electrode *2* increased; curve B, taken 26 seconds later, shows the potentials of the two spots almost balancing, as the injury beneath electrode *1* was disappearing and the injury beneath electrode *2* was not yet fully developed. Curve C shows the oppositely directed monophasic curve obtained 3 seconds later, as injury developed beneath electrode *2*. The durations of the monophasic responses in curves A and C happen to be the same (0.20 sec.) but the contours are different. Experiments such as these are convincing in showing the reversible character of the injury.

The contour of the monophasic electrogram found in these experiments resembled the curves in figure 2. The curve started with a rapid deflection followed in turn by a plateau and then by a more gradual return to normal. The primary deviation often had either a minor vibration preceding it, was notched, had a spike at or near the plateau level or showed several of these deviations. The plateau usually showed a tendency to deviate toward the base line; occasionally it was rounded, or moved away from the base line. The relative durations of these various portions of the curve were not constant for different regions of the same heart.

In measuring the duration of the monophasic curve, the preliminary dip, when it occurred, was ignored and the onset of the curve taken as the point where the curve begins to deviate in the direction it finally takes. The end point was taken as the point where the curve returns to the isoelectric level. Actual test has shown that in good curves (and only these were used in measurements) the error of selecting points and measuring the curve was ± 0.005 second. Deviations of 0.01 second or less are considered in subsequent curves as being within the experimental error of measurement.

In order to eliminate from the curves artifacts introduced by the method itself a number of preliminary experiments were done to determine the effect on duration and contour of *1*, varying the pressure on the electrode; *2*, varying the amount of moisture on the surface of the heart, and *3*, the effect of continued application of the electrode with optimum pressure for long periods of time.

It was found that once the electrode is applied to the heart with sufficient pressure to produce a monophasic curve, moderate increases in pressure had no effect on the duration or contour of the curve. Very heavy pressure, however, not only interferes with the movements of the heart but fails to give the constancy of contour and duration over long periods obtained with lighter pressure. The optimum pressure as measured crudely by applying equivalent pressure against a balance is roughly about 15 grams. When this optimum pressure is applied the monophasic curve can be maintained unchanged in contour and duration for as long as 30 minutes as shown by actual test.

When the heart surface was very wet, the end point of the curve (the point at which the descending limb returns to the base line) was more rounded and not so distinct, and the duration of the curve was as much as 0.02 of a second longer than that of the curve taken from the same spots when the heart surface was dry. It was in order to prevent the saline from the moist wick of the electrodes from running down onto the heart, that the yarns were soaked in a 0.3 per cent agar–0.9 per cent saline solution and allowed to dry partially. These wicks were found to hold enough moisture to serve as good conductors throughout the experiment with-

out dripping. It is important therefore to keep the moisture on the heart at a minimum in order to confine the response to as small a region around the electrodes as possible. Care must be taken, however, to avoid being too energetic in this regard. In one of these experiments we found that even the process of wiping the uninjured spot with gauze in order to remove excess fluid was sufficient to cause a temporary reversal in the direction of the monophasic curve.

EXPERIMENTS DEALING WITH THE SOURCE OF THE MONOPHASIC ELECTRICAL RESPONSE. Having developed a method by which a monophasic electrogram could be obtained of constant duration and contour over a period of a half hour, we next attempted to determine *1*, the influence of changing the injured region while keeping the lead off on the uninjured region unchanged; *2*, the influence of shifting the lead off to various uninjured regions while using the same injured region for the second lead off, and *3*, the effect of Na, Ca or K salts when applied on the injured and when applied on the uninjured area.

Changing the location of the injured area was easily accomplished simply by moving the special electrode to a new spot and producing a new region of injury. In the seven experiments in which this was tried, it was surprising to find that the duration of the monophasic curve was not altered within the experimental error of measurement by using different injury spots with the same uninjured area lead off (see table 1 and segments A, B and E of fig. 3). The injured areas were not confined to one ventricle but were located for the most part on the anterior surface of the heart. This observation is not in accord with Wilson's contention that the currents are generated at the injured area, since it would be surprising indeed that the duration should have been constant.

Changing the location of the uninjured lead off point did modify the duration of the monophasic electrograms as much as 0.03 of a second in some experiments (see table 2 and segments B, C, D and E of fig. 3). The uninjured lead off points selected were on various parts of the two ventricles, mostly on the anterior surface but some were placed on the lateral and posterior walls. A selector switch was used to quickly shift the connection of the injured lead off point with the various uninjured lead offs. In each instance, the connection of the injured lead off point with the first uninjured spot used was restored at the end of the series and another curve recorded to check the constancy of the contours. In each experiment reported in table 2 the duration of the original monophasic curve was the same at the end of the experiment within the error of measurement as at the start. Since moisture on the heart was kept constant and at a minimum in this series the variations, though small, are measures of real inequality in the time span of electrical activity in the various regions explored. They indicate that it is the uninjured and not the injured area

which determines the monophasic electrogram and are therefore not in accord with Wilson's contention.

The differences in the monophasic electrograms obtained in these experiments while small are nevertheless significant, considering the short duration of the invasion period of the ventricles in the dog as indicated by the short span of the QRS complex of the dog's electrocardiogram. They prove that the duration of electrical activity in the various fractions of the dog's heart are sufficiently unequal to account for the appearance of the T wave in the electrocardiogram as postulated by Katz. This inequality in duration of the electrical activity makes the pathway of retreat different from the pathway of invasion with the result that the T wave does not

TABLE 1

Effect on duration of monophasic electrogram of using lead offs from different injured regions with a lead off from the same uninjured region

EXP. NO.	NUMBER OF INJURED LEAD OFFS USED	RANGE OF VARIATIONS OF DURATION IN DIFFERENT MONOPHASIC CURVES
		sec.
3	2	0.01
4	3	0.00
5	2	0.00
6a	3	0.01
6b	3	0.01
7	3	0.01
8	2	0.01

Range in different experiments—0.00 to 0.01 second.

TABLE 2

Effect on duration of monophasic electrogram of using lead offs from different uninjured regions with a lead off from the same injured region

EXP. NO.	NUMBER OF UNINJURED LEAD OFFS USED	RANGE OF VARIATIONS OF DURATION IN DIFFERENT MONOPHASIC CURVES
		sec.
2	5	0.01
3	5	0.02
4	5	0.01
5	5	0.03
6a	5	0.02
6b	5	0.02
7	5	0.02
8	5	0.03

Range in different experiments—0.01 to 0.03.

have a fixed relationship to the QRS complex. It is for this reason that the T wave is not opposite in direction to the QRS complex. The variations in the duration of the electrical activity of the various fractions responsible for the electrocardiogram account for the variability of the T wave pattern in relation to the QRS complex in different animals.

The hypothesis that the monophasic curve registers the activity at the uninjured electrode and not at the injured was further supported by noting that solutions of Na, Ca and K of various tonicity altered the duration of the monophasic curve in most instances when applied to the uninjured area, but, without exception, had no influence when applied to the injured area. The results are summarized in table 3 and a typical set of curves is shown in figure 4. These experiments also emphasize the fact that the duration of electrical activity in various fractions of the heart can

be affected by chemical (or physical) factors operating in limited regions of the heart (cf. Katz, 2).

As a final test of the hypothesis that the monophasic curve registers the activity at the uninjured electrode we decided to compare the latent period of extrasystoles induced near the electrode on the injured region with that of extrasystoles induced near the electrode on the uninjured region. These extrasystoles were induced by electrical stimulation during cardiac standstill produced by vagal stimulation. The stimulus was registered as a sharp electric deflection; the time interval from this point until the appearance of the electrical deflection set up by the extrasystole was measured

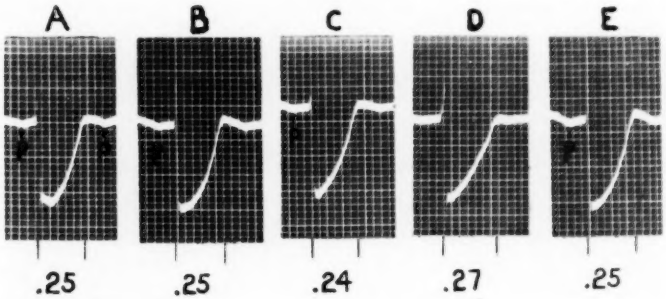

Fig. 3. Segments of monophasic curves obtained in a typical experiment (expt. 5, tables 1 and 2) to show the effect on the curve's duration of shifting the electrodes to different injured and uninjured regions. The preliminary small rounded depressions in each curve are due to auricular activity, *P*. The preliminary oscillations are extrinsic effects. The heart cycle is the same in all the curves. The onset and end of the monophasic curve is shown by the vertical black lines. The duration in seconds is given below each curve. Segment A shows the monophasic curve obtained from injured region 1 and uninjured region a; segment B, injured region 2 and uninjured region a; segment C, injured region 2 and uninjured region b; segment D, injured region 2 and uninjured region c; segment E (control at end of series) injured region 2 and uninjured region a. Discussed in text.

as the latent period. If the curve set up by the extrasystole was due to a potential arising in the injured area, we felt that the latent period would be shorter when the extrasystole was induced close to the injured area. However, as shown in table 4, the reverse was actually the case, indicating conclusively that the potential change recorded occurs at the electrode on the uninjured region.

It is interesting to note that when the electrode on the injured region is connected to a distant electrode on the hind leg, the latency of the extrasystole induced near the injured area is greatly reduced as compared with its duration when the connection is made on an electrode directly on an

uninjured region of the heart. This observation indicates that the assumption that the distant electrode is "indifferent" is not tenable. In reality the distant electrode picks up the effects of various regions of the heart and is "electrically nearer" in actual fact to the point of origin of the extrasystole than the electrode on the injured area of the heart. In no

TABLE 3

Effect of various salts on the duration of the monophasic electrogram when applied on the uninjured and injured regions

EXP. NO.	SALT SOLUTION USED AND CONCENTRATION	CHANGE IN DURATION OF MONOPHASIC CURVE WHEN APPLIED	
		On injured region	On uninjured region
		sec.	*sec.*
1	NaCl hypertonic		+0.03
2	NaCl hypertonic		+0.02
7	NaCl isotonic	0	0
8	KCl isotonic		0
	$CaCl_2$ isotonic		0
	NaCl isotonic	0	+0.02
9	KCl saturated		+0.05 (contour changed)
	$CaCl_2$ saturated		0
	NaCl hypertonic	0	+0.02
10	KCl saturated	0	−0.06
	$CaCl_2$ saturated	0	+0.01
	NaCl hypertonic	0	+0.02
11	KCl saturated	0	+0.02
	$CaCl_2$ saturated	0	0
	NaCl hypertonic	0	0 and +0.03 to +0.04
12	KCl saturated	0	(Curve became diphasic)
	$CaCl_2$ saturated	0	−0.02
Range in various experiments		0	0 to ±0.06

other way can we explain the shortening in latency of the extrasystole simply by moving the electrode away from the heart to a distant point. We have elsewhere expressed the view that the difference between electrodes on the heart and those at distance is the lack of advantage of particular regions in the latter case (Katz, 2). The electrode when at a distance picks up the currents of all parts of the heart and not of one region in particular as is the case when the electrode is on the heart.

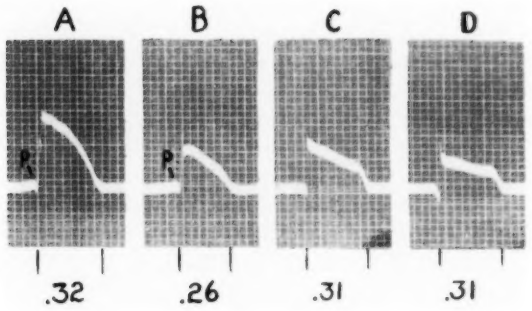

Fig. 4. Segments of monophasic curves obtained in a typical experiment (expt. 10, table 3) to show the effect on the curve's duration of applying a saturated KCl solution to the injured and uninjured regions. The auricular wave, P, just precedes the monophasic curves. The heart cycle is the same in all the curves. The onset and end of the monophasic curves is shown by vertical black lines. The duration in seconds is given below each curve. Segment A is a control showing the curve obtained from injured region 1 and uninjured region a; segment B is the curve obtained one minute after application of KCl to uninjured region a; segment C is a control showing the curve obtained from injured region 2 and uninjured region b; segment D is the curve obtained one minute after application of KCl to injured region 2. Discussed in text.

TABLE 4

Latent period between stimulation and the appearance of electrical deflection of the extrasystole when induced near lead off on injured and near lead off on uninjured area

EXP. NO.	DURATION OF LATENT PERIOD IN SECONDS WHEN EXTRASYSOLE WAS INDUCED		
	Near injured region (uninjured lead off on heart)	Near uninjured region	Near injured region (other lead off electrode on hind leg)
	sec.	*sec.*	*sec.*
11	0.06	0.03	0.035
12	0.065	0.035	0.0 0.015
13	0.05 0.06 0.06		0.03
Range	0.05 to 0.065	0.03 to 0.035	0.0 to 0.035

The facts presented here indicate clearly that the monophasic electrogram is a measure of the potential variation at the uninjured region and not

at the injured spot. They also substantiate the concept that the independent variability of the T wave in respect to the QRS complex can be attributed to variations in duration of the electrical activity of various parts of the ventricles.

In reviewing the subject of the genesis of the T wave one of us (Katz, 2) stated "that the T wave of the electrocardiogram is caused by the unbalancing of electrical stresses during the retreat of excitation which are in part the result of non-simultaneous onset of excitation, and in part the result of unequal duration of excitation in various fractions of the heart." The non-simultaneous onset of excitation is generally conceded by workers in this field. The present research has demonstrated that the duration of excitation is actually unequal in various fractions of the mammalian heart and can easily be altered. The evidence for the concept of the genesis of the T wave seems therefore complete. The possibility exists of course that other factors contribute to determine the T wave contour, such as for example, alteration of the contacts of the heart with the chest during contraction and relaxation of the heart. Nevertheless the simple concept outlined above is adequate to account for the T wave contour encountered and its relative lability without implicating mysterious processes and involved theories. In essence then the T wave appears to be due to electrical stresses set up by *1*, asynchronous stimulation of the ventricles, and *2*, unequal durations of excitation and the depolarized state in various fractions of the heart.

SUMMARY

1. A method is described by which persistent monophasic electrograms can be obtained in the mammalian heart. This consists essentially in leading off from the injured spot produced by moderate pressure on a special type of electrode.

2. It is shown that the monophasic response obtained by leading off from injured and uninjured spots of the heart measures the potential changes in the region of the uninjured electrode and not in the region of the injured electrode.

3. Sufficient variations were noted in the duration of electrical activity in different regions of the heart to support the concept of the origin of the T wave in the electrocardiogram advanced by Katz.

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THE RELATION OF THE ORGAN OF CORTI TO AUDIO-ELECTRIC PHENOMENA IN DEAF ALBINO CATS[1]

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In 1930 Wever and Bray first observed that stimulation of the ear by sound gave rise to changes of electric potential in the auditory nerve which reproduced the sound as it reached the ear. Since that time there has been considerable discussion as to whether this response in reality parallels the conscious phenomenon of hearing, or is simply an irrelevant electrical artifact. The answer to this question is of obvious importance in evaluating the increasing mass of data on the physiology of the ear which is being accumulated by this method. It was thought that some light could be thrown on the problem by applying such a technique to the study of the responses in animals known to be clinically deaf. Accordingly a strain of incompletely albinotic cats was selected. These are known in many instances to be deaf, and show a relatively simple and constant type of lesion which is limited to the inner ear (Alexander, 1900, 1904).

Initial experiments along this line indicated that the intactness of the organ of Corti is essential for the production of the changes in electric potential resulting from sound stimulation. These findings were presented by Howe and Guild to the American Association of Anatomists in April, 1933. Since that time a similar conclusion has been reached by Davis, Derbyshire, Lurie and Saul (1934), who have reported tests on one cat and one waltzing guinea pig. It now remains to elaborate somewhat the data on which the original report was based.

Twelve albino cats' ears have been tested with an apparatus already described elsewhere (Witting, 1932). Briefly, leads were taken from the auditory nerve, and in some cases also from the round window membrane. These were designed to pick up any electric disturbances which might be generated in the cochlea when voice-sounds and pure tones ranging from 180 to 8000 cycles were introduced into the ear. The currents thus obtained passed through five stages of vacuum tube amplification, to become finally audible to an observer as the sound of the stimulating frequency. A second circuit gave direct telephonic communication from the source of sound to the observer, so that it was a simple matter to compare the tele-

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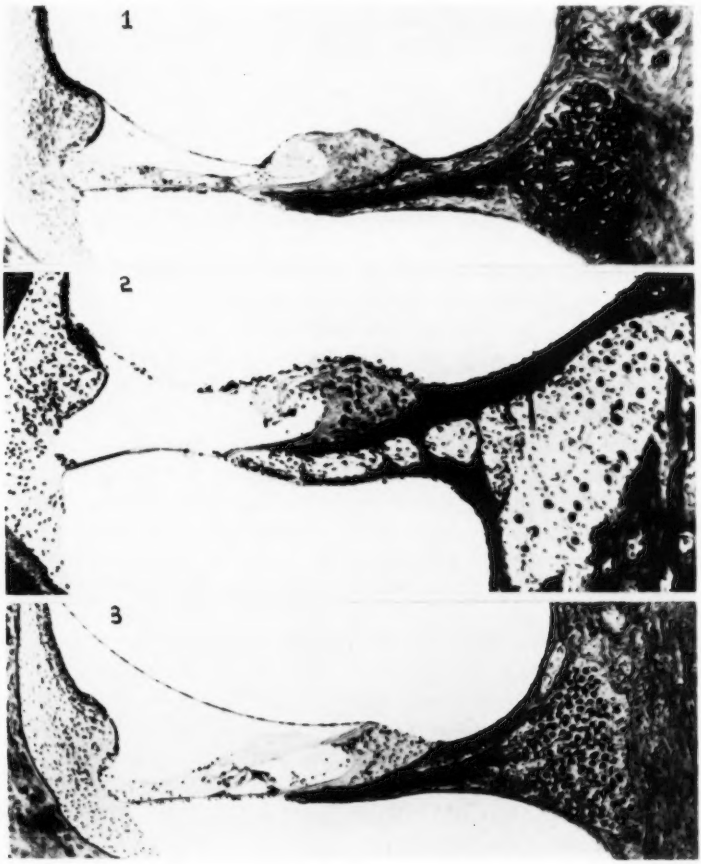
phonic reproduction with the same sound as it was transmitted through the cochlear apparatus of the cat. The amount of resistance required to reduce the responses through the direct circuit to the same intensity as the cochlear response could be conveniently read in decibels. It thus constituted a definite measure of the efficiency of the circuit in which the cat's ear was the only unknown element. With this apparatus it has been possible to obtain positive responses to sounds in hundreds of cases. Negative results in practically every instance are dependent upon lesions of the hearing apparatus. Breaks in the lead wires or other defects of the apparatus were, of course, carefully controlled.

Serial sections of the 12 ears tested for sound transmission revealed a marked similarity in the character of the lesions. The most striking features were restricted to the organ of Corti which showed a complete disappearance of hair cells, with atrophy and distortion of the supporting cells (fig. 1). In extreme cases (fig. 2) there was total destruction of all the elements, so that only the basilar membrane remained. At the same time the tectorial membrane was turned into the internal spiral sulcus to which in some regions it was adherent. The stria vascularis showed extreme shrinkage and loss of capillaries. There was more or less complete collapse of the cochlear duct, so that Reissner's membrane had come to lie upon the structures beneath it. While these changes in the organ of Corti and neighboring structures were always marked, there was in 3 ears only slight atrophy and disappearance of nerve fibers and cells in the spiral ganglion. The nerve lesions thus appeared to be secondary to those of the organ of Corti; in some cases a total destruction of the end organ was accompanied by a normal ganglion cell count (fig. 1). This phase of the problem is discussed in a separate communication (Howe, 1934). The remaining portions of the cochlear and vestibular apparatus were normal, with the exception of the sacculus, which was collapsed and showed atrophy of hair cells and supporting structures in its macula. The middle ear showed no lesions whatever.

The physiological tests were chiefly concerned with five cats in which this condition was bilateral. Prior to the experiment these animals had shown no reactions to any sounds whatever. Under experimental conditions no electrical responses were detected from either auditory nerve or round window membrane, although in some instances tones of more than the usual intensity were employed. Checks of apparatus were naturally most scrupulous.

The objection has been raised that the electrical responses of the cochlea are due to microphonic effects of the middle ear, vibrations of electrodes, mechanical and electric effects from other tissues. Such experiments as the above automatically dispose of such possibilities, since electrical transmission fails in these ears when the only abnormality is restricted to the

cochlea. In most of the animals there were large numbers of normal-appearing nerve fibers so that direct mechanical stimulation of nerve may



Figs. 1–3

Fig. 1. Portion of albino cat's cochlea showing atrophy of organ of Corti. No sound transmission. Van Gieson stain. × 80.

Fig. 2. Portion of albino cat's cochlea showing complete disappearance of organ of Corti. No sound transmission. Hematoxylin and eosin. × 89.

Fig. 3. Portion of albino cat's cochlea from side opposite that of figure 2. Normal appearance. Normal sound transmission. Hematoxylin and eosin. × 67.

be dismissed as a cause of the electrical phenomena. In these experiments Reissner's membrane was out of its usual position—a condition which may

be cited as an argument that, acting in some theoretical capacity as a condenser, it had a part in the generation of the electric impulse. Certain unpublished observations on non-transmitting ears of other types indicate, however, that this structure may be found in its normal relations without the production of any potential changes from sound stimulation. One can say then with certainty that the integrity of the organ of Corti is essential for the production of the electrical phenomena.

A slight variation is seen in a sixth cat where the cochlear lesions were unilateral. The right side was normal from every point of view (fig. 3), while serial sections of the left showed maximal degenerative changes in the organ of Corti (fig. 2). The animal had exhibited no signs of deafness previous to the test. In view of the foregoing experiments there can be little doubt that the left ear was completely functionless, yet it was possible to pick up 500 cycles from the nerve on that side. It seems definite that this was due to a crossed bone conduction effect from the good side. Such a crossing can readily take place if the vibrating source of sound happens to touch the animal's skull.

Davis et al. (1934) have distinguished in the cat's ear two electrical phenomena—the response of the cochlea which is obtained at its greatest intensity from the round window membrane, and which constitutes a simple sine wave reproduction of any stimulating frequency. This may also be recorded from the auditory nerve where, in addition, it is possible to distinguish by their technique a "true nerve action current" which conforms more exactly to known responses in other peripheral nerves. The observations here recorded do not attempt to distinguish between these two effects, although it seems likely that the currents recorded were largely the response of the cochlea. The significant observation lies in the fact that no sounds of any character were transmitted either from nerve or round window when the cochlea was sufficiently damaged to give the picture of clinical deafness. One concludes then that the electric currents obtained from the cochlea in response to sound are dependent upon the integrity of the organ of Corti, and may legitimately be regarded as an index of the ear's normal activity.

SUMMARY

Six albino cats have been tested for audio-electric responses from the cochlear nerve when pure tones were introduced into the ears. In no case where the animal was clinically deaf was any electrical effect obtained, although in hundreds of normal cases it was readily demonstrable. The cochlea of these albino cats differed from the normal in that there was a characteristic atrophy affecting principally the organ of Corti. It seems justifiable therefore to ascribe the production of the electrical response to the activity of sensory cells in the organ of Corti.

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A STUDY OF THE VARIATIONS IN THE SACCHAROGENIC POWER OF HUMAN SALIVA

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As early as 1897 attempts were made to determine the variation in the ptyalin content of human saliva by measuring its saccharogenic or its amyloclastic power. Since that time the variation among different individuals, and in the same individual at different times of the day has been studied by a number of investigators (1). In these previous studies no attempt was made to prevent inactivation of the ptyalin from the time the saliva was collected until it was added to the substrate, and in only one case (1) was the hydrogen ion concentration of the substrate controlled. It is not surprising, therefore, that their results show a distinct lack of uniformity. In view of these facts this investigation was undertaken with the object of measuring under rigidly controlled conditions the saccharogenic power of the saliva secreted by different individuals at the same time of day, and by the same individual at different times during the day. The rate of secretion as well as the total solids content of the saliva was also determined. Since no work has been reported on the effect of the ingestion of coffee on the activity of saliva, a few experiments of this nature were carried out.

EXPERIMENTAL. Five subjects, all healthy young women, were used in this investigation. They were on a normal mixed diet without tea or coffee. No food was taken between meals although water was drunk as desired except during the half hour preceding collection.

Collection and preservation of saliva. Since it has been shown by Chittenden and Richards (2) that mechanical stimulation affects salivary activity, the saliva was collected with as little motion of the jaws as possible while the subject was sitting quietly. The secretion was discharged into a weighing bottle surrounded by ice. The time required for the collection was recorded and the sample weighed. It was immediately and rapidly filtered by suction to remove food particles and other debris, and was kept surrounded by ice in an electric refrigerator until needed. Just before using, the saliva was diluted with 24 parts of redistilled water. Since the samples collected before meals were not used until from two to two and one-half hours later, it was of course necessary to ascertain whether stand-

ing under these conditions affected the activity of the enzyme. Several experiments performed with this in view showed little or no appreciable deterioration.

Determination of salivary activity. The saccharogenic power of the saliva was determined by Sherman's method (3). One cubic centimeter of diluted saliva was allowed to act for one-half hour at 40° on 100 cc. of a 1 per cent dispersion of soluble starch to which had been added, as activators, 5 cc. of 1M NaCl and 2.5 cc. of 0.02M disodium hydrogen phosphate. The pH value of the substrate, determined colorimetrically, was adjusted to 6.4 by means of 0.01N NaOH. At the end of one-half hour the action of the enzyme was stopped by the addition of 50 cc. of Fehling's solution to each flask. These were immediately immersed in a boiling water bath for fifteen minutes and the weight of cuprous oxide formed by the reducing sugar in this length of time was taken as a measure of the saccharogenic power of the saliva.

Determinations of activity were made on samples collected immediately before breakfast, lunch and dinner and one-half hour after each meal. This covered a twelve hour period from 7:30 a.m. to 7:30 p.m.

Results. The results of the before and after breakfast determinations show that a considerable increase in salivary activity occurred during the period of the meal and the half hour following. In seventeen experiments performed on five individuals the average increase was 77 per cent, and in some instances the activity was more than doubled. Appreciable increases in rate of secretion and total solids content of the saliva were evident. It was further observed that although a subject consumed the same kind and quantity of food on successive mornings, the increase in enzymic activity on some days was several times that on others. There were marked variations among different individuals in rate of secretion, saccharogenic power, and total solids content of the saliva.

The saliva collected after lunch was likewise more active than the before lunch secretion. This increase, however, was less marked than that noted in the case of breakfast, the average increase being 27 per cent. There was a similar increase in rate of flow, but the variations in total solids content were irregular.

After dinner an average increase of 11 per cent in saccharogenic power was observed with small but appreciable increases in both rate of secretion and total solids content.

Table 1 shows the average values obtained throughout the day for rate of secretion, saccharogenic power, and percentage of total solids. From a consideration of these data, it appears that the lowest value for the twelve hour day is recorded before breakfast, the highest after dinner. The enormous increase in activity shown one-half hour after breakfast is followed by a decline during the forenoon. There were several individual excep-

tions to this, however. Then a 27 per cent increase in saccharogenic power due to the ingestion of food at noon and a smaller but still appreciable increase of 11 per cent after dinner is noted. During the twelve hours between 7:30 p.m. and 7:30 a.m. there is obviously a considerable loss in activity. The data on rate of secretion show that the variation in this value follows the same general trend as does the variation in saccharogenic power. Except for the irregularity of a decrease after lunch, for which we have no explanation, the average figures for total solids content show a gradual increase during the day.

A few determinations were made of the starch digesting power of samples of saliva secreted before and after breakfasts at which two cups of fairly strong coffee had been consumed. Two tests each were made on two subjects the results of which show no decrease in salivary activity following the ingestion of coffee. The increases are greater than the average increase

TABLE 1*

Variations in the saliva during the day

	RATE OF SECRETION IN GRAMS PER MINUTE	SACCHAROGENIC POWER IN MGM. Cu_2O	TOTAL SOLIDS
			per cent
Before breakfast	0.66	71	0.35
After breakfast	0.89	125	0.40
Before lunch	0.76	101	0.43
After lunch	0.88	128	0.39
Before dinner	0.83	124	0.43
After dinner	0.89	138	0.47

* The values recorded are the averages of all determinations.

for the same subjects on a coffee free diet, but not greater than might occur in a daily variation.

SUMMARY

1. There is a marked variation in the saccharogenic power of the saliva of different individuals on mixed diets when the samples are collected at the same time of day.

2. The variation in the activity of samples collected from the same individual on successive days is appreciable.

3. The ingestion of food at mealtime is followed by increased activity of the salivary amylase. This increase is greatest with breakfast, least with the evening meal. There is usually a small decline in activity between breakfast and lunch, and lunch and dinner, the substantial loss occurring during the night.

4. The variation in rate of secretion of the saliva follows qualitatively but not quantitatively the variation in its saccharogenic power.

5. The evidence for variation in total solids content indicates that this factor also qualitatively parallels the activity of the saliva. Results on this point, however, show irregularities.

6. The ingestion of coffee with breakfast does not diminish the activity of saliva collected after breakfast.

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VAGO-INHIBITORY EFFECTS ON THE RESPIRATORY METABOLISM OF THE HEART AFTER TREATMENT WITH DINITROPHENOL[1]

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In previous communications the authors have shown that there is a significant reduction of the respiratory metabolism of non-beating turtle's auricles when inhibited by vagus stimulation (Garrey and Boykin, 1933, 1934). It was desired to determine what might be the effect upon this phenomenon of intensifying the oxidation processes, since it was uncertain whether this vagal effect might not be obscured or entirely eliminated by the augmented rate of oxygen utilization; on the other hand there existed the possibility that the inhibitory effect might be proportional to the increased oxygen consumption.

Effects of dinitrophenol. It is now well known that 2–4 dinitrophenol increases tissue oxidations and this effect has been demonstrated on excised frog muscle *in vitro* by Ehrenfest and Ronzoni (1933). Our first experiments were directed to determining whether and to what extent this drug would affect the respiratory metabolism of non-beating turtle's auricles. The procedure was as follows. The hearts were removed from the body and all the ventricular tissue removed; the sinus venosus was then carefully dissected away until all contractions of the auricles ceased. The auricular preparation was washed thoroughly with bicarbonate buffered Ringer's solution (pH 7.6) and soaked in the solution for about one hour, after which the preparation was placed on a gauze sponge to remove excess fluid and transferred to a Barcroft-Warburg apparatus in which it was submerged in 2 cc. of Ringer's solution. The apparatus was filled with oxygen, and the oxygen consumption was computed from manometric readings with corrections determined by blank control manometers. After determining the normal rate of oxygen consumption in Ringer's solution for varying periods of time and noting that the oxygen consumption progressed at a constant rate, a known quantity of dinitrophenol was tipped into the Ringer's solution from a side arm of the vessel, so that the concentration of the drug was 0.5 mgm. per cent.

[1] This investigation was financed by a Fluid Research Fund granted by the Rockefeller Foundation.

In a series of sixteen such experiments conducted during the early summer months at room temperatures, using the auricles of Chelydra serpentina and Pseudemys elegans, the resting oxygen consumption averaged 191 cu. mm. per hour per gram (moist weight), while in the dinitrophenol solution it averaged 512 cu. mm. The minimal *increase* was 159 per cent, the maximal was 425 per cent, and over half of the auricles showed a rate of oxygen consumption which was approximately three times that of the normal resting preparation. The high rate of respiratory metabolism after treatment with dinitrophenol is further exemplified by the high oxygen utilization shown in table 1. Automatic beats were not observed in any of the preparations.

Vagus inhibition. It was next determined that vagus inhibition could be effected while a sinus-auricle preparation was under the influence of dinitrophenol. To this end the sinus and auricles with their intact vagi were removed from the animal and a suspension preparation made to record on a kymographion. After determining the functional integrity of the vagi with the preparation in Ringer's solution, the solution was replaced by the desired concentrations of dinitrophenol dissolved in Ringer's solution. The concentrations varied from 0.5 mgm. per cent to 1.5 mgm. per cent. In these solutions the contractions continued for several hours but progressively decreased in height. Vagus stimulation produced prompt and typical inhibition throughout the entire period, in one instance after the drug had acted continuously for twenty hours at room temperature. The inhibition affected both rate and force of contractions and there was evidence that the inhibitory mechanism was actually more sensitive than in the normal preparation.

Vagus effects on augmented oxygen consumption due to dinitrophenol. With the above facts established we proceeded to determine the effects of vagus inhibition on oxygen consumption. The technical procedures in preparing the auricles were essentially those previously described by the authors (1934). The sinus-auricle-vagus preparation was soaked in the desired concentration of dinitrophenol (1.5 and 0.5 mgm. per cent) in Ringer's solution for fifteen minutes or more. The preparation was transferred to gauze to remove excess of the solution and cardiac standstill was secured by painting a strong solution of acetylcholine over the entire sinus venosus. The preparation was mounted on a paraffined wire frame in the Barcroft-Warburg chamber and the functioning vagi threaded to the outside for stimulation as previously described (1934). Such preparations show augmented respiratory metabolism in an atmosphere of oxygen for several hours although all our determinations of oxygen consumption were made during the first two hours. The results of twenty complete experiments on as many hearts are summarized in table 1.

In every instance there was a distinct decrease in the oxygen consump-

tion of the auricles during inhibition although only one vagus was stimulated and maximum effects therefore were not induced. The individual differences tabulated are to be ascribed in part to differences in functional effectiveness of the vagi resulting from the dissection. The actual decrease in oxygen consumption is of the same order of magnitude as that

TABLE 1

Inhibitory depression of oxygen used by resting turtles' auricles during respiratory augmentation while treated with dinitrophenol

Oxygen consumption expressed in cubic millimeters per hour, per gram (moist weight). The right vagus only was stimulated. Room temperature.

RESTING BEORE INHIBITION	DURING INHIBITION	ACTUAL DECREASE	PERCENTAGE DECREASE	RESTING AFTER RECOVERY
			per cent	
860	820	40	4.6	860
880	780	100	11.36	870
570	530	40	7.00	590
570	492	78	13.68	590
755	662	93	12.31	778
722	535	187	25.90	810
666	540	126	18.91	696
*				
568	507	61	10.74	594
476	457	19	4.00	473
480	387	93	19.37	442
360	313	47	13.05	368
295	257	38	12.88	340
480	424	56	11.67	446
750	686	64	8.54	730
600	530	70	11.66	604
520	458	62	11.92	540
540	395	145	26.85	503
656	614	42	6.40	659
658	548	110	16.20	650
492	430	62	8.75	512
Average. 594.9	518.2	78.2	12.78	602.7

* Dinitrophenol concentration above the line was 1.5 mgm. per cent, below the line it was 0.5 mgm. per cent.

previously reported for normal hearts under similar conditions, the percentage decrease however is less owing to the high rate of metabolism. The maximum decrease of 26 per cent would correspond in the normal heart to a decrease of something like 75 per cent—which is nearly the maximum decrease reported by us for normal preparations. The average decrease in respiratory metabolism was 12.78 per cent; this likewise would

correspond to a decrease of about 38 per cent if calculated for normal hearts, based on the experimentally determined probability that the average oxygen consumption of normal hearts is only one-third that of hearts under the influence of the concentrations of dinitrophenol used in these tests.

CONCLUSION. The experiments afford a clear demonstration of the fact that the increase in respiratory metabolism induced by dinitrophenol does not prevent the inhibitory depression of oxidations; this was the case even in those instances in which the oxidations were increased to five times the normal rate. Quite as striking is the fact that the inhibitory effects upon oxygen consumption are not proportional to the rate of tissue oxidations but seem to be of a constant order of magnitude whether the respiratory metabolism is normal or augmented.

These facts arouse a speculative interest in the nature of the processes which make up the sum total of cellular respiration as we have encountered it in cardiac muscle. If one assumes that the chemical processes culminating in oxygen consumption proceed as a series of simple catenary reactions, one can postulate that if inhibitory processes break the chain at any point the ultimate effect would be a reduction in oxygen consumption which presumably would be proportional to the intensity of the respiratory metabolism. It was found however that this was not the case, which makes it seem more probable that the reactions may progress along two different lines one of which may be looked upon as the primary process which is essentially and fundamentally augmented by the action of dinitrophenol, a process which is unaffected by the physical alterations which accompany the inhibitory effects of vagus stimulation. The other process may be considered as of a secondary order, ancillary in character but readily depressed by inhibition, a process however which may assume a large rôle in the resting respiration of cardiac muscle as is demonstrated by the large extent to which it may be depressed by maximal inhibition induced by strong stimulation of both vagi.

The character of the reactions in these two oxidative trends, assuming that they may ultimately be demonstrated, remains to be determined. It is significant however that Ehrenfest and Ronzoni (1933) have demonstrated a marked acceleration of anaerobic lactate production caused by dinitrophenol and that lactic acid also accumulates in muscle under aerobic conditions in spite of the high oxygen consumption. These facts are thus directly related to what we have referred to as the primary or direct-line oxidations. These phenomena however are not concerned with the processes of inhibition and presumably therefore are not related to the depression of those oxidations which occur during inhibition. This statement is based on evidence which has already been obtained, and which is accumulating, that inhibition, at least insofar as concerns its physical manifesta-

tions, may be induced not only when there is an accumulation of lactic acid in the cardiac muscle but also when no lactic acid is being formed.

SUMMARY

Dinitrophenol augments the respiratory metabolism of resting cardiac muscle *in vitro*, the rate of oxygen consumption being increased three-fold, on the average, and in some instances as much as five-fold. Vagus inhibition is still effective under these conditions and a decrease in oxygen consumption by resting turtle's auricles accompanies vagus stimulation. This decrease was not proportional to the intensified oxidations but was of the same order of magnitude as that previously found for normal resting auricles. It is suggested that cellular oxidations may be the resultant of two different chemical trends, only one of which is normally affected during inhibition and that this effect is only a by-product of inhibition but is not an essential part of its mechanism.

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THE EFFECT OF OESTRIN INJECTIONS UPON THE DEVELOPING OVA OF MICE AND RABBITS[1]

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While purified oestrin is apparently ineffective in terminating pregnancy after the implantation of embryos (Gostimirovic, 1930; Levin, Katzman and Doisy, 1931; D'Amour, D'Amour, and Gustavson, 1933) it seems certain that injection in the early stages effectively prevents gestation. D'Amour (1934) describing the histological effects of oestrin injection during pregnancy finds definite effects upon the oviducts of rats during the pre-implantation period, e.g., "hyperplasia of the uterine mucous membrane, a considerable degree of fibrosis, usually glandular hyperplasia and an increase in the number of ciliated cells in the Fallopian tube. The fluid filling the uterus is thin and watery . . . . and resembles the fluid found during oestrus." He states further that the post-implantation effect is probably directly upon the fetus, and the implication therefore seems to be that pre-implantation prevention of gestation is due to an inhibition of the proper type of tissue growth in the oviducts with no direct effect upon the blastoderms. The data of this paper are concerned with a direct examination of the ova in the oviducts of mated mice and rabbits which received daily injections of oestrin for various periods of time after copulation.

The mice used in these experiments were examined twice daily for vaginal plugs, and daily injections of 5 r.u. of oestrin (Parke-Davis Theelin[2]) were begun ordinarily on the same day that the plug was found (*cf.* table 1). The ova were obtained from the tubes by the method described by Gilchrist and Pincus (1932), especial care being taken in these experiments to include all of the tube which is known to enter the uterus below and at one side of the apex (Lee, 1928). Further, the lumen of each uterine horn was flushed with Ringer's solution and if six or more days had elapsed since copulation each horn was split longitudinally and carefully examined for possible implantations.

[1] These investigations were in part supported by a grant from the National Research Council Committee for Problems of Sex.

[2] We are indebted to Dr. O. Kamm of the Parke-Davis Co. for generous supplies of theelin.

TABLE 1

The development of the early embryos of control and oestrin-injected mice

MOUSE NUMBER	TIME INTERVAL AFTER MATING	TOTAL RAT UNITS OESTRIN INJECTED	RECENT CORPORA LUTEA IN OVARY*	FOUND IN TUBES OR UTERUS†	NUMBER AND CONDITION OF OVA
			Control series		
75	30 hours		P	T	6 segmenting ova, varied in development
76	36 hours		P	T	1 ovum, normal
84	4 days		P	U	4 ova, showing segmentation cavity (fig. 1)
85	4 days		A		Not pregnant
78	6 days		P?	U	5 degenerating ova in left horn (fig. 2)
88	6 days		A		Not pregnant
77	7 days		A		Not pregnant
86	7 days		P	U	7 ova showing typical elongation (fig. 3)
87	10 days		A?	T	Fragmenting ova in left tube (fig. 4)
			Injections begun day of copulation		
15	28 hours	10	P	T	5 ova, 2 in two-cell stage, three in one-cell (fig. 5)
12	35 hours	10	P	T	5 ova, in four-cell stage
11	2 days	10	P	T	7 ova, 8 to 12 cells
32	2 days	10	P	T	6 ova, 6 to 16 cells (fig. 6)
13	3 days	20	P?	T	2 clear ova (?) apparently yolkless
10	3½ days	20	P?	U	2 ova in left horn only, one degenerate, one with doubtful segmentation cavity
17	4 days	25	P	T	1 ovum, early morula (fig. 7)
18	4 days	25	P	T	3 ova, morula stage
69	8 days	35	P	T	3 ova, degenerate
16	8 days	45	P	T	7 ova, 32 cells to morula
1	10 days	50	P	T	6 ova, varied but all showed degeneration
8	10 days	55	P	T	4 ova, degenerate (fig. 8)
31	12 days	60	P	T	7 ova, degenerate ("mulberry" ovary) (fig. 9)
4	12 days	65	P	T	10 ova, degenerate
26	14 days	75	P	T	5 ova, degenerate (fig. 10)
			Injections begun one day after copulation		
32	2 days‡	10	P	T	6 ova, up to 16-cell stage (fig. 6)
29	4 days	40	A		Not pregnant
31	12 days	60	?	T	7 ova degenerate ("mulberry" ovary) (fig. 9)
30	13 days	60	?		Not pregnant
33	9 days‡	80	A		Not pregnant
28	13 days‡	120	?		Not pregnant

* P = present; A = absent.

† T = tubes; U = uterus.

‡ Received 10 r.u. daily.

Since it is well known that mouse eggs enter the uterus from the 3rd to 4th day after mating and become implanted on the 6th to 7th days (*cf.* Enzmann, Saphir and Pincus, 1932; also Hartman, 1932) only a small series of control mice were examined. These data are included in table 1, with the data on injected animals.

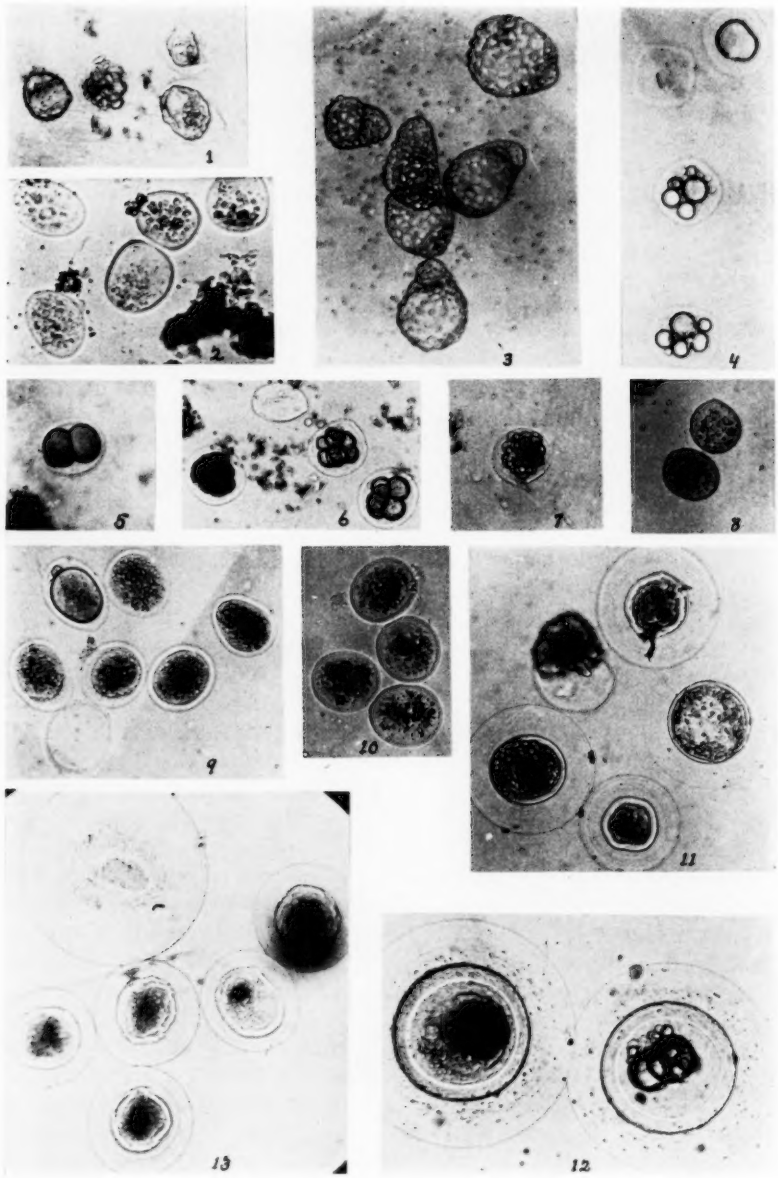
The most obvious feature of the data of table 1 is the finding of ova in the tubes only, except in one animal, even when autopsies were conducted long after the time uterine blastocysts are normally found. There are two possible explanations of this observation: 1, the blastocysts never enter the uterus but remain locked in the tubes where they eventually degenerate; 2, the blastocysts enter the uterus but disappear with great rapidity, and all eggs found in the tubes after the 4th day post coitum are the products of an ovulation succeeding the one occurring at the time of mating. The latter conclusion seems to be supported by the findings of *a*, Pincus (1930) that unfertilized (or undeveloped) ova do not remain long in the uterus; *b*, Deansley (1931) who found that the oestrus induced by similar oestrin doses during pseudopregnancy in the mouse is sometimes accompanied by an ovulation, and *c*, of ova in the uterus of mouse 10 (see table 1) at $3\frac{1}{2}$ days post coitum, e.g., at the critical period for the entry of eggs into the uterus. Deansley's cases were, however, relatively few and her, as well as most of the work on continuous oestrin injection seems to indicate that ovulation is usually inhibited. Furthermore, practically all of the ova recovered in the tubes after the third day showed evidences of previous segmentation even when quite degenerate (figs. 7 to 10), and the fragmentation of tubal ova in the mouse, which ordinarily does not begin until two days after ovulation, is not quite like the degeneration here observed (*cf.* Charlton, 1917; and fig. 4).

The obvious solution of these difficulties resides in employing an animal that does not ovulate spontaneously. Accordingly we have made similar oestrin injections into rabbits mated to fertile males. In this species ovulation occurs only at mating or under the influence of gonad-stimulating hormones, oestrin does not cause ovulation, and cleavage-like fragmentation in the tubes is rare (Pincus, 1930). Fertilized ova enter the uterus with great regularity between the 70th and 80th hours after copulation (Gregory, 1930; Pincus, 1930) and fertilization is ordinarily completed by the 12th to 13th hours after copulation (Pincus and Enzmann, 1932). Mated does were injected intraperitoneally once a day with 100 to 150 rat units of oestrin, the first injection being given at 12 to 13 hours after copulation in order to avoid the chance of any interference with the fertilization of the ova.

The results of the rabbit experiments are tabulated in table 2.

It is evident from the data of table 2 that the ova of oestrin-injected rabbits remain in the Fallopian tubes for at least several days beyond the

PLATE 1



Figs. 1–13

normal term. The finding of ova in the uterus of some of the animals may be due to individual variation in response to the particular oestrin dosage employed. The injection of 100 r.u. per diem was more or less arbitrarily chosen, and it is quite possible that sufficiently large doses would effectively prevent all ova from entering the uterus.

If we may deduce that essentially the same result is had both in mice and in rabbits, namely, the prevention of the passage of the ova from tube to uterus, a somewhat anomalous picture results of the nature of the processes involving the transport of ova. In certain polyoestrus animals, e.g., the sow, the monkey, and man, the post-oestrus period during which the ova are in the tubes is characterized by the presence of large contraction waves in the excised Fallopian tubes in contrast with the presence of small, less powerful contractions in the interoestrum and pregnancy (Seckinger, 1923; Seckinger and Corner, 1923; Seckinger and Snyder, 1926). It has been suggested that these powerful contractions are responsible for oval transport and that they are due to the action of oestrin. In the intact uterus of the rabbit the period immediately following ovulation is characterized by the change from a state of relatively powerful contraction to one of relatively weak contraction (Reynolds, 1932). Unfortunately Reynolds' observations are confined to uterine contractions, but he has adequately demonstrated that the powerful contractions are due to oestrin action, the less powerful ones to progestin action (Reynolds and Allen, 1932). If the tubes of the rabbit behave as do the uteri then the data for the rabbit are in marked contrast to those for polyoestrous animals. The dosage of oestrin employed on the rabbits in our experiments is well below any amount which may affect the contractions of the rabbit uterus during pseudopregnancy (*cf.* Reynolds and Allen, 1932). Unless the threshold for an

Plate 1. Explanation of figures. Microphotographs of material recovered from control mice.

1. Blastocysts from uterus four days after plug.
2. Degenerating ova recovered from uterus six days after plug.
3. Blastocysts from uterus seven days after plug.
4. Fragmenting ova from tubes ten days after plug.

Microphotographs of material recovered from experimental mice.

5. Developing embryo thirty hours after plug.
6. Developing embryos fifty hours after plug.
7. Developing embryo four days after plug.
8. Degenerating ova ten days after plug.
9. Degenerating embryos twelve days after plug.
10. Degenerating embryos fourteen days after plug.

Microphotographs of material recovered from rabbits.

11. Developing embryos recovered from right cornu five days after copulation, development delayed.
12. Degenerating embryos recovered from left tube five days after copulation.
13. Degenerating embryos recovered from right tube seven days after copulation.

oestrin effect upon tubal contraction in the rabbit is remarkably lower than the uterine threshold it is doubtful that the effects observed in these rabbits is due to a change in the nature of the tubal contraction. Since the effects of oestrin injection appear to be the same in both mice and rabbits we are inclined to believe that either 1, a closure of the tubo-uterine junction is effected, or 2, the nature of ciliary movement is altered.

TABLE 2

The development of the early embryos of oestrin injected rabbits

RABBIT NUMBER	DAYS AFTER MATING	TOTAL RAT UNITS INJECTED	RIGHT OR LEFT OVIDUCT	NUMBER OF CORPORA LUTEA	FOUND IN TUBES OR UTERUS	NUMBER AND CONDITION OF OVA
1	4	500	Right	3	T	1 ovum recovered, with inner cell mass, many blood cells
			Left	2		No ova recovered, too many blood cells
5	5	500	Right	3		No ova recovered, much blood
			Left	3	U	3 ova; one nearly normal in growth stage, 2 degenerating gastrulae
3	5	600	Right	4	U	4 ova; segmentation cavity and inner cell mass; development definitely delayed (fig. 11)
			Left	4	T	3 ova; delayed development
4	5	850	Right	1	T	1 ovum; degenerate
			Left	3	T	2 ova, one degenerate, one fragmented (fig. 12)
2	7	600	Right	5	T	5 ova; degenerate; (albumen covered débris present)
			Left	5	T	5 degenerate ova; (albumen covered débris) (fig. 13)
6*	7	600	Right	4	T	3 ova, degenerate but showing evidences of cleavage
			Left	5	T	4 ova, as in right tube
7	9	800	Right	4	T & U	1 ovum in lower end of tube, 3 ova in uterus; degenerate
			Left	4	U	4 ova; degenerate

* Subcutaneous injections.

While the prevention of uterine entry is at first sight somewhat surprising an often overlooked observation of Long and Evans (1922) is perhaps significant. They found that the ova of non-pregnant female rats degenerate in the tubes and do not enter the uterus. The unmated female rat is notable for having no functional luteal phase unless pseudopregnant or pregnant. Wislocki and Snyder (1933) found that the ova of an ovulation induced in the rabbit by prolan during pregnancy, descended into the uterus 12 hours earlier than normally. It will be noted that these ova

were traversing the tubes in the presence of large persisting functional corpora lutea in addition to the corpora lutea of their own ovulation.

The inference then seems to be that oestrin inhibits the passage of tubal eggs and progestin (or some luteal substance) accelerates this passage.

These experiments do not adequately demonstrate the possible direct effect upon the embryos of oestrin injections. Fairly complete evidence can be had from various types of discontinuous injections now in progress. But we believe that the simple retention of ova in the Fallopian tubes is probably not the factor responsible for the observed degree of degeneration because 1, tubal pregnancies certainly occur on occasion in the rabbit, and 2, ova removed from the uterus of injected animals show degenerative changes comparable to those occurring in the tubes (fig. 11).

The possibility exists that the effects observed are due to local irritation of the oviducts by the intraperitoneal injections. The data on rabbit 6 (table 2) who received subcutaneous dosages at a site remote from the oviducts make this possibility remote.

We are indebted to Dr. E. V. Enzmann for technical assistance and advice.

CONCLUSIONS

1. Daily injections of oestrin begun on the day of mating ordinarily result in the retention of ova in the Fallopian tubes in both mice (5 r.u. per day) and rabbits (100–150 r.u. per day).

2. All ova, whether they descend into the uterus or remain in the tubes, show definite signs of degeneration by the fourth day after copulation.

3. These observations indicate, therefore, that the prevention of pregnancy resulting from oestrin injections during the early preimplantation period is due *a*, to the degeneration of the ova long before they are capable of implantation, and *b*, secondarily to the prevention of uterine entry of the blastocysts.

4. The retention of the ova in the tubes is probably due to a closure of the tubo-uterine junction as a result of oestrin action, although an inhibition of the normal ciliary mechanism is not excluded.

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THE DISTRIBUTION OF EXCITATION AND INHIBITION FOLLOWING SYMPATHETIC STIMULATION OF THE LARGE INTESTINE

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The fact that throughout the gastro-intestinal tract excitatory as well as inhibitory processes are set up on stimulation of sympathetic nerves is established by the more recent work in which depressant anesthetics have not been used (Thomas, 1926; McSwiney, 1931; Templeton and Lawson, 1932; Garry, 1934). In the dog's colon there seems to be a temporal separation of the antagonistic processes, depression appearing as the primary response, excitation as a delayed response (Lawson, 1934a). A similar temporal separation has been observed in the small intestine (Thomas, 1926), and in the stomach (Oser, 1892; Weitz and Vollers, 1926).

Recovery from the primary inhibitory response to adrenalin or to stimulation of the lumbar colonic or the hypogastric nerve usually occurs first in the uppermost responding segment of the colon. Since the inhibitory phase seems to be cut short in each segment by the motor process, a possible interpretation of the peristaltoid sequence of recovery is that there is a spatial as well as a temporal separation of the antagonistic processes set up by the stimulus, excitatory processes predominating in the upper segments, inhibitory processes in the caudal segments.

The peristaltoid nature of the response to these stimuli is the more striking in view of the fact that spontaneous motility in the dog's colon is not consistently peristaltic. The myenteric reflex is not so easily elicited as in the small intestine (Bayliss and Starling, 1899; Garry, 1934). Temporally and spatially the excitatory and the inhibitory response to sympathetic stimuli are such as to suggest that these stimuli are actually peristaltogenic, or that a peristaltoid gradient of irritability (Alvarez, 1928) is established by the stimulus. Indirect evidence has already been offered that the centrally-isolated inferior mesenteric ganglia function to maintain the relative irritability of adjacent colon segments (Lawson, 1934).

METHOD OF STUDY. Dogs were barbitalized and prepared for observation by exposure of the viscera in a saline bath as previously described (Lawson, 1934 a and b). Simultaneous records were taken of the longi-

tudinal activity of the proximal, middle and distal segments (records 1, 2, and 3 in all the figures). The internal anal sphincter was also recorded with a condom sphincterograph (record 4 in the figures).

The lumbar colonic and the hypogastric nerves were divided to remove the influence of the inferior mesenteric ganglia. The colon was usually decentralized as far caudally as the pelvic plexus by stripping the right colonic vessels, transecting the ileum, and dividing the mesocolon near its root. In seven animals complete decentralization was done by also transecting the colon between ligatures close to the external sphincter and dividing all pelvic nerves and blood vessels. The results obtained in the completely decentralized preparations and in those of which the proximal and middle segments were decentralized were in essential agreement with the results obtained in the smaller number (about one-fourth) in which preliminary decentralization was limited to post-ganglionic division of the lumbar outflow.

Evidence for a peristaltoid activation of intersegmental pathways was sought by comparing the response to a constant stimulus before and after interrupting nervous pathways between segments. Enteric pathways were interrupted by transecting the colon between recorded segments, ligation of the segments being omitted to lessen trauma. The segments were loosely sutured together after separation to preserve their mechanical interrelationships. Mesocolic pathways were interrupted by a transverse division of the mesocolon between recorded segments, the paracolic artery and vein being stripped in the course of the incision and carefully preserved. Usually within fifteen to thirty minutes after either procedure spontaneous motility became constant, and there was no further progressive change in the response during the following period of two to four hours. Responses elicited during the first half-hour, during which progressive changes suggested the presence of traumatic stimulation, are not included in any of the results.

The constant dose of adrenalin was from 2 to 4 cc. of a 1:50,000 solution. The constant lumbar colonic stimulus consisted of one minute of faradization of the peripheral stump of the nerve in a fluid electrode (Lawson, 1934b), the secondary coil of the inductorium being set usually at 5 cm., and the primary activated by three dry cells. With stimuli of this strength and duration the response to this nerve throughout the colon is usually similar to the response to adrenalin.

The constant stimulus, either adrenalin or lumbar colonic stimulation, was applied from two to six times after each interruption, at intervals of 15 to 30 minutes, and the responses superimposed for averaging. The construction of group average responses for each experimental condition from these individual averages has been described (Lawson, 1934a). Persistence of contractions superimposed on the tone change during the response

is indicated by lines which show maximum and minimum motility levels connected, for the sake of legibility, by conventionalized contraction waves. The group averages also give the average maximum and minimum limits of spontaneous motility during the five-minute period preceding each stimulation.

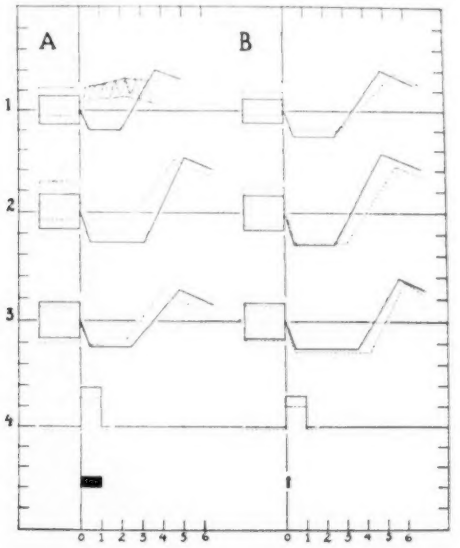

Fig. 1. Group averages. Control responses with peripheral plexuses intact are shown as solid lines. The broken lines give the responses after division of the mesocolon between segments 1 and 2. A. Faradization of the lumbar colonic nerve, 23 animals. B. Injection of adrenalin, 19 animals. Thirteen animals appear in both groups. The average limits of motility during the five-minute period preceding stimulation are given by the rectangles erected on the zero time axis in each section. The abscissae express time in minutes from the beginning of stimulation, the ordinates centimeters of tracing height from the base line. Only the primary motor phase of the sphincter response is shown.

RESULTS. The findings which lend themselves to a semi-quantitative presentation are summarized in the group averages (figs. 1 to 4) and in table 1.

Ascending influences. Division of the mesentery between either the proximal and the middle, or the middle and the distal segment always abolished the inhibitory response to the lumbar colonic nerve in the colon above the interruption (figs. 1 A and 2 A). The motor response of the immediately superadjacent segment was not abolished, however, but now appeared as the primary response (fig. 5). Incomplete data suggest that

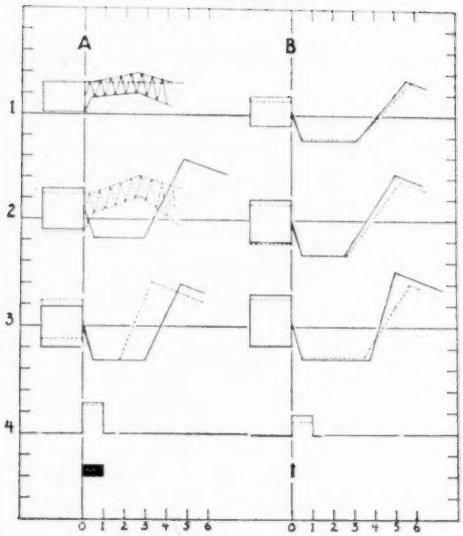

Fig. 2. Group averages. Control responses with the mesenteric plexus interrupted between segments 1 and 2 are shown as solid lines. The broken lines give the responses after division of the mesentery between segments 2 and 3. A. The response to the lumbar colonic nerve, 11 animals. B. The response to adrenalin, 5 animals. Three animals appear in both groups. The construction of the figure is the same as in figure 1.

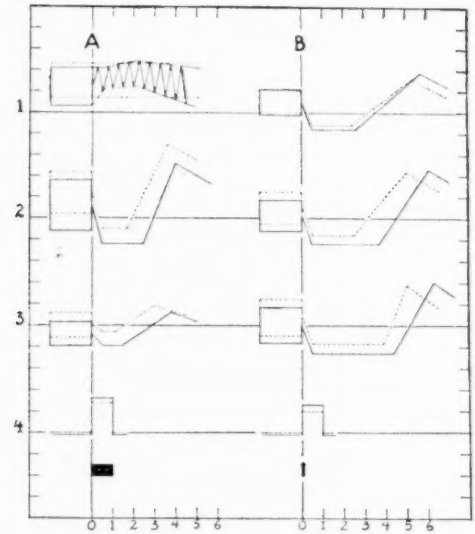

Fig. 3. Group averages. Control responses with the mesenteric plexus interrupted between segments 1 and 2 are shown as solid lines. The broken lines give the responses after transection of the colon between segments 1 and 2. A. The response to the lumbar colonic nerve, 8 animals. B. The response to adrenalin, 11 animals. Five animals appear in both groups. The construction of the figure is the same as in figure 1.

the excitatory response of the mesenterally denervated cranial segment, which is a constant finding, is of higher amplitude if the proximal colon is not decentralized.

That the primary excitatory response of the cranial segment is due to processes ascending the gut, and not to incomplete mesenteric interruption of lumbar colonic fibres is shown by the abolition of the response on subsequent division of the colon at the same level (figs. 3 A and 4 A). Further, these excitatory processes ascend the gut for only a limited distance, since

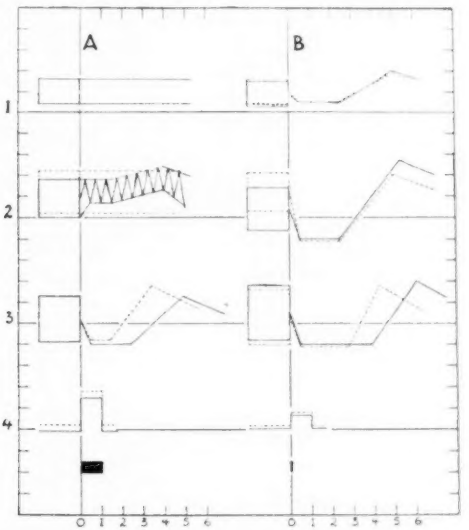

Fig. 4. Group averages. Control responses with the mesenteric plexus divided between segments 1 and 2, and between segments 2 and 3, and with the colon transected between segments 1 and 2, are shown as solid lines. The broken lines give the response after transection of the colon between segments 2 and 3. A. The response to the lumbar colonic nerve, 9 animals. B. The response to adrenalin, 9 animals. Seven animals appear in both groups. The construction of the figure is the same as in figure 1.

the motor response of the cranial segment was abolished by a second mesenteric interruption from ten to fifteen centimeters lower, the segment just cranial to the second interruption now showing the excitatory response (fig. 2 A).

The response to adrenalin in the colon above the level of mesenteric interruption gives no evidence that division of the mesentery increases the excitatory irritability or reduces the inhibitory irritability of the cranial segment (figs. 1 B and 2 B). Individual tracings show variable modifica-

tions in the response of the upper segment to adrenalin (compare fig. 5 B with fig. 6 B).

The excitatory influences ascending the gut on lumbar colonic stimulation may be due to lumbar colonic efferents, excitatory in function, which ascend by this route. Similar excitatory influences seem to ascend the gut following adrenalin injection, however, since division of the colon at any level consistently reduced the height of the motor response to adrenalin in the cranial segment (figs. 3 B and 4 B).

TABLE 1

The effect of interrupting the peripheral plexuses upon the length of the inhibitory phase

The effect is expressed by the number of animals in each column whose average response shows the modification.

CONTROL	EXPERIMENTAL PROCEDURE	SEGMENT	LUMBAR COLONIC				ADRENALIN			
			−	+	0	Total	−	+	0	Total
Intact	Division M 1-2 (fig. 1)	1	23*	0	0	23	5	10	4	19
		2	17	2	4		5	12	2	
		3	11	8	4		7	12	0	
M divided 1-2	Division M 2-3 (fig. 2)	1	—	—	—	11	2	1	2	5
		2	10*	0	1		0	1	4	
		3	8	1	2		3	2	0	
M divided 1-2	Transection C 1-2 (fig. 3)	1	—	—	—	8	5	2	4	11
		2	7	1	0		8	1	2	
		3	5	2	1		8	2	1	
M and C divided 1-2, M divided 2-3	Transection C 2-3 (fig. 4)	1	—	—	—	9	2	0	7	9
		2	—	—	—		2	3	4	
		3	8	0	1		6	0	3	

M = mesentery; C = colon. The level of interruption lies between the two segments named.

* Motor response.

Descending influences. Division of the mesentery at either the upper or the lower level produced equally striking modifications in the response to the lumbar colonic nerve in segments below the interruption. The inhibitory phase in the subjacent colon was consistently shortened, and the height of the excitatory response of the sphincter reduced (figs. 1 A and 2 A). When weak stimuli were employed, the inhibitory phase in the subjacent segments and the excitatory response of the sphincter were almost abolished. In a series of descending interruptions there was a step-like reduction in the primary inhibitory response of the caudal segment, and the primary excitatory response of the sphincter.

The modification seems to be due primarily to the modified response of the proximal segments, since the response to adrenalin in the segments below a mesenteric interruption was either not changed (fig. 2 B) or was changed in the opposite direction (fig. 1 B). Yet the activity descending the gut from the mesenterally denervated cranial segment, as shown by subsequent division of the colon, seems to be quite independent of the

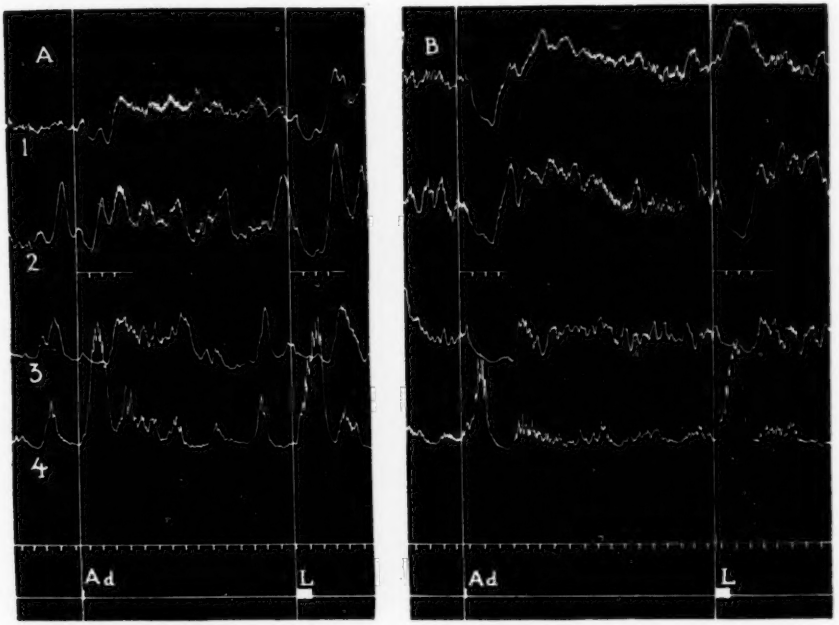

Fig. 5. Original tracing. Dog 56, weight 18 kgm. Response to faradization of the lumbar colonic nerve *L*, and to intravenous injection of 4 cc. adrenalin 1:50,000, *Ad*. A. Control responses with peripheral plexuses intact. B. One and one-half hour after division of the mesocolon between segments 1 and 2. The time interval between sections A and B is approximately 2 hours. Time in minutes.

muscular response of the cranial segment. The inhibitory response in the caudal segments to both adrenalin and the lumbar colonic nerve was reduced, as though inhibitory influences were descending from the cranial segment independently of the sign of its primary muscular response (figs. 3 and 4). This finding may suggest that traumatic excitatory processes are set up by the colon transection which invalidate the use of this procedure for the demonstration of descending intersegmental influences. The

evidence against the introduction of significant traumatic artefacts to account for the shortening of the caudal inhibitory response is indirect: 1. The modified response remains constant for as long as four hours after the enteric interruption (figs. 6 and 8). 2. The shortening of the inhibitory response in the subjacent segment is usually just sufficient to abolish the difference between the response in the two separated segments (figs. 3 B and 4 B). 3. There is no constant relationship betwen the effect of the procedure on spontaneous motility and the effect on the response (fig. 4 A, segment 3; fig. 4 B, segments 2 and 3; fig. 6 D and E). 4. Enteric

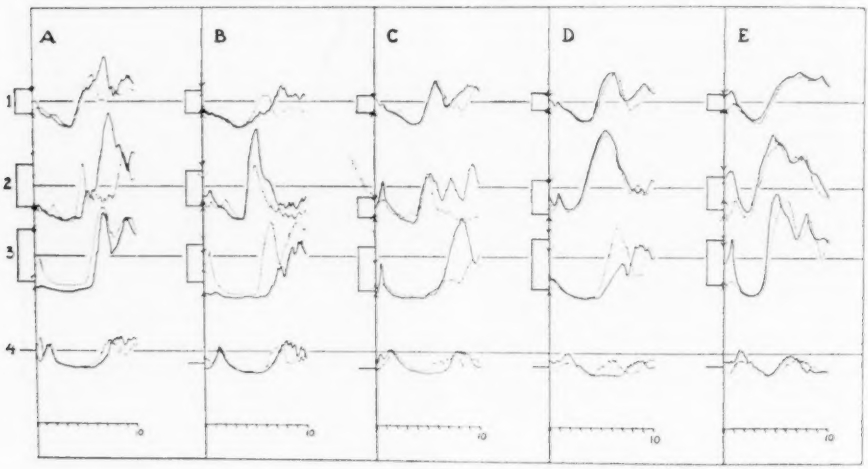

Fig. 6. Superimposed responses. Dog 53, weight 7.2 kgm. Response to intravenous injection of 2 cc. adrenalin, 1:50,000. The solid line is the first, and the dotted line the second response in each period. The time interval between injections in any period is 20 minutes. The construction of the figure is the same as in figure 8. A. Control responses with peripheral plexuses intact. B. After division of the mesentery between segments 1 and 2. C. After division of the mesentery between segments 2 and 3. D. After transection of the colon between segments 1 and 2. E. After transection of the colon between segments 2 and 3.

interruption seems not to produce these modifications in the response without preliminary mesenteric interruption (fig. 7).

The activation of descending enteric pathways by the lumbar colonic nerve. In order to test directly for descending enteric processes set up by the lumbar colonic nerve, the response of the distal segment and the sphincter were recorded in six animals after interruption of all descending mesenteric fibres for the caudal region of the colon at the primary bifurcation of the nerve as it enters the paracolic plexus. The irritability of the lower segments to adrenalin was not significantly modified. In these animals the

only striking change in the response to the lumbar colonic was the introduction of a weak primary motor phase in the distal segment (fig. 8). This was followed by both an inhibitory phase and a delayed motor phase. The response of the distal segment was thus triphasic, like that of the sphincter. The response of the sphincter, on the other hand, was only quantitatively modified from its intact control. The inhibitory phase in both the sphincter and the distal segment was reduced by interrupting the mesentery between the proximal and the middle segment, and abolished by dividing the colon at the same level. After thus limiting descending

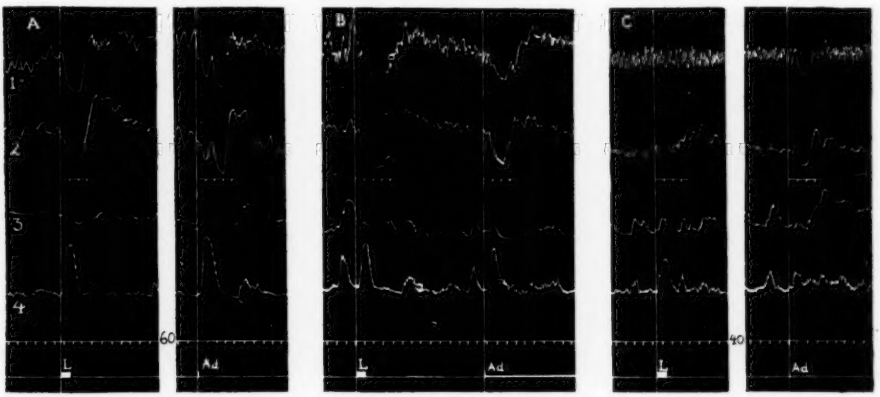

Fig. 7. Original tracing. Dog 67, weight 8.7 kgm. Response to faradization of the lumbar colonic nerve, *L*, and to intravenous injection of 2 cc. adrenalin 1:50,000, *Ad*. A. Control responses with peripheral plexuses intact. B. Forty-five minutes after transection of the colon between segments 1 and 2, and between segments 2 and 3. C. Sixty minutes after division of the mesocolon between segments 1 and 2. The time interval between lettered sections of the tracing is approximately 2 hours. Time in minutes. Time is also marked in minutes from the beginning of stimulation below the record of segment 2.

processes, lumbar colonic stimulation elicited a high, monophasic, excitatory response in both the distal segment and the sphincter (fig. 8 D). The persistent response of the distal segment and sphincter after mesenteric denervation was shown to be due to enterally transmitted processes descending from the segments above by its abolition after dividing the colon at the bifurcation of the lumbar colonic, or after interrupting all ascending fibres at that level.

The rôle of the mesocolic plexus. In only one procedure is there conclusive evidence for the activation of fibres in the mesocolic plexus other than lumbar colonic efferents. In the response to adrenalin the consistent in-

crease in the inhibitory response in subjacent segments, and reduction in the primary excitatory response of the sphincter, following interruption of the proximal mesocolon (fig. 1 B) suggests the removal of descending influences, transmitted by the mesenteric plexus, which are predominantly excitatory to both the colon and the sphincter. The relative inefficacy

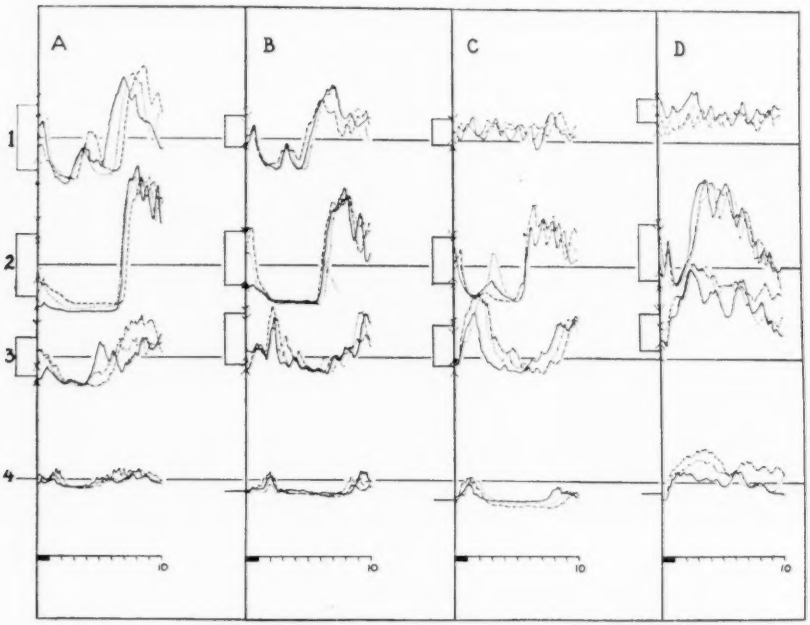

Fig. 8. Superimposed responses. Dog 62. Response to faradization of the lumbar colonic nerve. The solid line is the first, the fine broken line the second, and the coarse broken line the third response in each period. The time interval between responses in any period is 15 minutes. The limits of motility during the five-minute period preceding each stimulation are marked by darts on the zero time axis, and the average limits are drawn as rectangles. A. Control responses with the peripheral plexuses intact. B. After division of the mesenteric plexus below the primary bifurcation of the lumbar colonic nerve (above segment 3). C. After division of the mesenteric plexus between segments 1 and 2. D. After transection of the colon between segments 1 and 2. Time in minutes from the beginning of stimulation in each period. The control motility levels of period A are projected as reference abscissae.

of a second interruption lower in the mesentery (fig. 2 B) may mean that a center similar in function to the inferior mesenteric ganglia is located in the proximal mesocolon. There is, however, a reduction in the motor phase in the distal segment and the sphincter following the second inter-

ruption, which may be of significance. Descending activity in the mesenteric plexus is also suggested by the finding, in a preliminary series of 11 animals, that division of the proximal mesocolon after the colon has been divided shortens the inhibitory response to both adrenalin and the lumbar colonic in the subjacent segments (fig. 7). Descending activity in the mesocolic plexus thus seems to be inhibitory in the response to the lumbar colonic whether the colon is intact or not, but in the response to adrenalin the sign of the descending mesenteric influences seems to depend upon the integrity of enteric pathways. Enteric interruption, without mesenteric interruption at the same level, produced variable modifications, or none at all, in either response. The suggested interdependence of the two pathways requires further investigation.

Discussion. The evidence for activation of enteric pathways by the lumbar colonic is direct. This nerve is stated to be distributed by way of the mesentery (Garry, 1934), and insofar as the primary inhibitory response of the colon and excitatory response of the sphincter serve as an index to the functional integrity of the nerve, the present data support this statement. With the mesenteric distribution of the nerve limited to the middle segment, however, stimulation still evokes responses in remote segments both above and below, which are abolished by division of the colon. The response above is excitatory, the response below both excitatory and inhibitory, with the inhibitory response predominating except in the sphincter area. The difference in the response in the cranial and the caudal segments to these enteric processes may be due either to specific differences in intrinsic irritability in the caudal and the cranial segments, or to a specific polar distribution of stimuli.

The latter interpretation is supported by the finding that after all intersegmental connections have been divided, segmental differences in the response to adrenalin are practically abolished. If there are differences in the irritability of the various segments to sympathetic stimuli, these findings suggest that they are extrinsically determined. Even the sphincter, whose primary excitatory response to sympathetic stimuli in the intact colon bears a reciprocal relationship to the primary inhibitory response of the rest of the colon, is at least in part dependent upon intersegmental connections for its specific response.

The response to adrenalin both above and below a complete interruption of intersegmental pathways is modified as though the interruption had abolished ascending excitatory and descending inhibitory processes (excitatory to the sphincter) identical with those set up on lumbar colonic stimulation. Other interpretations are incompatible with the change observed in spontaneous motility levels, and the change observed in the same animals in the response to the lumbar colonic. The simplest interpretation of all the data is that adrenalin activates enteric pathways in the

same manner as lumbar colonic stimulation. The apparent identity of the ascending and descending enteric processes operative following these two dissimilar modes of stimulating the sympathetic terminal apparatus suggests that ascending enteric excitation and descending enteric inhibition are secondary to any stimulation of the sympathetic mechanism.

The change in spontaneous motility following an interruption of intersegmental pathways gives no evidence that ascending excitatory and descending inhibitory processes are present during unstimulated motility. Although additional data on the spontaneous activity of these intersegmental pathways under more carefully controlled conditions are required, the suggestion is warranted that the activity of such pathways is modified at least quantitatively by sympathetic stimuli. Absence of correlation between the change in spontaneous motility following modifying procedures, and the change in the response to sympathetic stimuli has been previously reported (McSwiney and Robson, 1931; Lawson, 1934). No close correlation would be expected if sympathetic stimuli set up intersegmental influences, excitatory or inhibitory in effect, which are not operative during spontaneous motility.

In the light of these findings the peristaltoid recovery of the colon from sympathetic inhibition assumes a new significance. Excitatory processes set up by the stimulus predominate in the cranial segments, inhibitory processes in the caudal segments. It is significant that the classical inhibitory response of the colon and excitatory response of the sphincter (Langley and Anderson, 1895; Elliott and Smith, 1904) are both progressively reduced as descending intersegmental influences are reduced. The more recently demonstrated delayed excitatory response of the colon (Templeton and Lawson, 1931; Lawson, 1934a) may be due entirely to ascending excitatory processes. Similarly, the motor effect of the lumbar colonic nerve on the distal segment, and the delayed inhibitory response of the sphincter to sympathetic stimuli (Lawson, 1934a) seem to be accounted for by activation of descending enteric pathways in the caudal end of the colon. The persistence of both excitatory and inhibitory responses in the isolated segment may mean, in the light of these findings, that in these relatively long segments intrasegmental influences similar to the demonstrated intersegmental influences are still operative following sympathetic stimulation of the segment.

Since every response which has been observed thus seems to depend, at least in part, upon a specific polar activation of enteric pathways by the stimulus, it is suggested that such polarization of the enteric apparatus is an essential function of the sympathetic supply to the large intestine. The dependence of the muscular response to sympathetic stimuli upon a great variety of dissimilar factors (McSwiney, 1931; Rosenblueth and Cannon, 1934) is the more readily understandable if, as these data on the colon

suggest, a variable enteric system is either interposed between the sympathetic nerve and the muscle, or is activated along with the latter. Whether polar activation of the enteric apparatus is the primary response to sympathetic stimuli, or whether such activation is a concomitant response with that of the muscle is a subject for investigation. The present data suggest only that enteric activation is not secondary to the muscular response, since there seems to be no close relationship between the muscular response of a segment and the influences ascending or descending from it.

The polarity of the intersegmental influences points to a nervous rather than a humoral mode of transmission, especially since intrinsic regional differences in the response to such a humoral agent as adrenalin are insignificant. The abolition of all response to the lumbar colonic nerve in completely denervated remote segments constitutes additional evidence against humoral influences, which is not, however, conclusive since in the denervation enteric humoral pathways are interrupted.

SUMMARY AND CONCLUSIONS

The activity of enteric intersegmental pathways following sympathetic stimulation of the decentralized or partially decentralized colon has been investigated by comparing the response to intravenous adrenalin or to stimulation of the lumbar colonic nerve before and after interruption of pathways in the colon and the mesocolon.

Direct evidence for activation of enteric pathways is offered by the finding that after limiting the mesenteric distribution of the lumbar colonic nerve to a restricted segment, stimulation of the nerve evokes responses in remote segments both above and below, which are abolished by division of the colon. The cranial segment responds with excitation; the caudal segment responds in a complex manner, the predominant response being inhibitory except in the sphincter area. Pure excitatory responses in the caudal segment are obtained after further limiting the cranial extent of the system reached by the stimulus.

After interruption of all intersegmental connections, the peristaltoid sequence of recovery from adrenalin inhibition is destroyed, the inhibitory phase terminating almost simultaneously in all the colon segments. The change in the response seems to be due to the abolition of ascending excitatory and descending inhibitory processes similar to those set up on lumbar colonic stimulation. The descending processes are excitatory to the internal anal sphincter.

The dependence of the local response in every segment upon ascending and descending enteric influences which do not seem to be spontaneously active, but which seem to be set up by both types of stimulation, suggests that a function of the sympathetic supply to the colon is the polar activation of the enteric apparatus, or the establishment of gradients of irritabil-

ity. The sympathetic colonic mechanism under these experimental conditions seems to be essentially peristaltogenic.

Preliminary data are presented which suggest activity in the centrally-isolated mesocolic plexus governing the relative irritability of adjacent colon segments.

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THE INCREASE IN INSULIN SECRETION FOLLOWING INJECTION OF EPINEPHRINE AND ITS RELATION TO THE HIGH LIVER GLYCOGEN VALUES OBTAINED[1,2]

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The following experiments were carried out for the purpose of studying the behavior of insulin secretion following injection of epinephrine, and to determine its possible relation to the high liver glycogen values obtained. It is evident that the increase in liver glycogen following several hours after injection of epinephrine is a secondary effect of the hormone (Cori, 1931), the primary effect being to bring about hydrolysis of hepatic glycogen. Zunz and La Barre (1927a, b) have demonstrated an increased insulin secretion following injection of epinephrine in dogs. If this extra insulin is responsible for the rise in hepatic glycogen, an animal having a constant supply of insulin adequate for normal purposes, which cannot be increased, would be expected to show a decrease in liver glycogen rather than an increase after injection of epinephrine.

METHOD. Dogs were used in these experiments. Some were depancreatized and given insulin (Lilly) subcutaneously in sufficient dosage to keep the blood sugar normal. Control experiments were done on normal dogs and on dogs which were depancreatized and allowed to become diabetic.

The first experiments reported (table 1) were on two female dogs (litter mates) which were not anesthetized. One had been depancreatized previously under ether and was given insulin twice daily. Both dogs lay quietly on a table during the entire day of an experiment while blood samples were taken at intervals from the femoral artery. Injection of insulin and epinephrine (Parke-Davis' adrenalin chloride 1:1000) subcutaneously did not disturb the depancreatized dog, though the normal one was sometimes made restless by epinephrine.

The remainder of the experiments were acute. The dogs were fed chopped raw beef for at least a week and then fasted 24 hours before an

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[2] We wish to thank Dr. H. E. Himwich for his criticisms and help in preparing this manuscript.

experiment. Amytal anesthesia was used in all cases. Some were depancreatized, and in the others the abdomen was opened and the pancreas manipulated. In most of the experiments the two vagi were cut in the

TABLE 1

Experiments on two littermate dogs showing the greater effectiveness of epinephrine in raising the blood sugar level when the animal cannot augment its insulin secretion

EXPERIMENT NUMBER	ANIMAL	INITIAL BLOOD SUGAR	FINAL		INSULIN DOSE		EPINEPHRINE DOSE	
			Hours: minutes after initial blood sugar	Blood sugar	Hours: minutes after initial blood sugar	Units subcutaneously	Hours: minutes after initial blood sugar	Subcutaneously
		mgm. per cent		*mgm. per cent*				*mgm. per kgm.*
1	Dog 1. Normal	89	5:50	90				
2	Dog 1. Normal	102	6:30	280			0:10	0.12
							4:25	0.12
3	Dog 2. Depancreatized	95	5:45	175	−4:55	2.0		
4	Dog 2. Depancreatized				−4:40	2.0		
		73			0:35	0.5		
			6:00	70	3:40	0.5		
5	Dog 2. Depancreatized				−4:30	2.0		
		83			0:50	0.5		
			5:53	90	4:20	0.5		
6	Dog 2. Depancreatized				−4:25	2.0		
		95			0:40	0.5	0:15	0.043
			6:05	390	4:35	0.5		
7	Dog 2. Depancreatized				−4:30	2.0		
		75			0:50	0.5	0:15	0.025
			6:15	243	4:20	0.5		
8	Dog 2. Depancreatized				−4:30	2.0		
		80	6:10	275	0:50	0.5	0:15	0.025
					4:25	0.5		

neck. This made no significant difference in the liver glycogen. Injection of epinephrine was by the subcutaneous route except where indicated. Samples of blood were drawn from the femoral artery and the blood sugar determined by the method of Folin and Wu (1920).

At the end of the experiment the dog was bled by cutting the carotid arteries and duplicate samples of liver and of the right semi-tendinosus muscle were obtained for analysis of glycogen. The time from the commencement of bleeding until the last sample was put into hot KOH was about 2 minutes. Glycogen was determined according to Pflüger's method as modified by Good, Kramer and Somogyi (1933). Somogyi's (1926) method of sugar determination was used in the analysis of the glucose formed by acid hydrolysis of glycogen. All values given for glycogen are averages of duplicate determinations.

TABLE 2

Normal dogs given epinephrine: amytal anesthesia

EXPERIMENT NUMBER	SEX	INITIAL BLOOD SUGAR	FINAL VALUES				EPINEPHRINE		REMARKS
			Hours: minutes after epinephrine	Blood sugar	Liver glycogen	Muscle glycogen	Hours: minutes after first dose	Subcutaneously	
		mgm. per cent		*mgm. per cent*	*mgm. per g.*	*mgm. per g.*		*mgm. per kgm.*	
9	♀	92	6:27	141	16.12	1.5		0.3	
10	♀	87						0.3	Vagi cut
							5:30	0.1	
			7:28	200	13.78	0.53	6:30	0.1*	
11	♂	95	2:41	200	11.47	3.9		0.3	Vagi cut; pancreas handled; old dog
12	♀	143	6:44	130	13.75	Trace		0.3	Vagi cut; pancreas
							2:45	0.3	handled
13	♂	96	5:21	215	4.48	1.95		0.3	Vagi cut; pancreas
							3:00	0.3	handled
Averages...		103		177	11.92	2.00			

* Intraperitoneally.

RESULTS. Table 1 shows that in a depancreatized dog receiving enough insulin to keep the blood sugar at or slightly below normal (expts. 4 and 5) it takes only $\frac{1}{10}$ the dose of epinephrine to raise the blood sugar to within the same range (expts. 7 and 8) that it does in the normal animal (expt. 2). Experiment 3 shows that this dog was completely depancreatized since failure to give insulin results in a rise of the blood sugar level.

In the acute experiments it was found that normal dogs under amytal anesthesia showed normal blood sugar levels (80–110 mgm. per cent), and that at the end of 6 to 8 hours their liver glycogen values ranged from 3.88 to 8.68 mgm. per gram liver, and muscle glycogen from 2.38 to 7.07 mgm. per gram muscle. In dogs which were depancreatized and kept under

amytal anesthesia, the blood sugar level consistently rose to near 200 mgm. per cent in 6 to 8 hours after the operation and the ranges of glycogen values under these conditions were: liver, 2.9 to 6.27 mgm. per gram; muscle, 5.4 to 7.77 mgm. per gram.

Table 2 shows the effect of epinephrine in normal dogs. In general, the result is a high liver glycogen (except in expt. 13, the explanation of which is not known), and a low muscle glycogen. The fact that the muscle

TABLE 3

Depancreatized dogs given insulin and epinephrine; amytal; vagi cut

EXPERIMENT NUMBER	SEX	INITIAL BLOOD SUGAR	FINAL VALUES				INSULIN DOSE		EPINEPHRINE DOSE	
			Hours: minutes after epinephrine	Blood sugar	Liver glycogen	Muscle glycogen	Hours: minutes after pancreatectomy	Subcutaneously	Hours: minutes after pancreatectomy	Subcutaneously
		mgm. per cent		*mgm. per cent*	*mgm. per g.*	*mgm. per g.*		*units per kgm.*		*mgm. per kgm.*
14	♀	103	6:32	116	5:05	0.64	−0:45	0.030	1:03	0.2
							1:00	0.060		
							2:14	0.030		
							3:34	0.030	4:21	0.2
							6:10	0.030	7:04	0.2*
15	♀	87	6:30	189	6.28	0.61	−0.47	0.042	1:00	0.3
							1:42	0.042		
							4:22	0.042	4:40	0.3
16	♂	89	5:35	215	1.26	1.08	−1:03	0.033	0.35	0.3
							1:10	0.017		
							3:22	0.033	4.00	0.3
17	♂	93	4:44	229	1.84	5.45	−1:29	0.033	0.17	0.2
							0:31	0.017		
							2:51	0.033	3:51	0.1
18	♀	85	7:15	196	1.86	1.99	−0:35	0.044	0:14	0.2
							1:46	0.017		
							4:18	0.033	3:32	0.3
Averages.....		92		189	3.26	1.95				

* Intraperitoneally

glycogen is not so low in experiment 11 as in the others is explained by the short duration.

In table 3 are the results obtained on depancreatized dogs given insulin and epinephrine. It had been previously determined that the insulin dosage as given in experiments 16, 17 and 18 would keep the blood sugar of a depancreatized dog within normal limits; and that in such dogs the liver and muscle glycogen were also within the limits cited above for nor-

mal animals. It will be noted that in table 3 the liver glycogen is low as compared with that of normal animals given epinephrine (table 2). In fact, it is considerably below normal except in experiments 14 and 15 in which cases the insulin dosage was greater than in experiments 16 to 18. In experiment 14 the low final blood sugar following epinephrine administration is no doubt explained by the large insulin dosage. Muscle glycogen is as low in these experiments as in normal dogs given epinephrine, except for experiment 17, where the epinephrine dosage was less than in the others.

DISCUSSION. The experiments referred to in table 1 show clearly the increased secretion of insulin which follows injection of epinephrine into the normal dog. It took ten times as much epinephrine in this animal to raise her blood sugar level close to the 300 mgm. per cent mark as it did in the depancreatized dog, even though it was clear that the amount of insulin the latter was receiving would ordinarily have kept her blood sugar below normal. This confirms Zunz and La Barre (1927a, b) who have already shown by cross-circulation experiments that injection of epinephrine into a normal dog brings about an increased secretion of insulin.

The liver glycogen values obtained in normal dogs receiving epinephrine (table 2) are above the normal level, while those of the depancreatized-insulinized dogs which were injected with epinephrine (table 3) are mostly well below normal. This observation points to the fact that in the normal animal epinephrine (or epinephrine hyperglycemia) stimulates an extra secretion of insulin which is responsible for the high liver glycogen values obtained.

Cori (1925) and Cori, Cori and Buchwald (1930) say that the increased liver glycogen following epinephrine is largely formed from lactic acid and that insulin does not accelerate the formation of liver glycogen from lactic acid. It is possible that the extra insulin acts indirectly, however, in bringing about increased liver glycogen formation. It is known that insulin does cause an increase in muscle glycogen if hypoglycemia is avoided (Best, Hoet and Marks, 1926; Cori and Cori, 1929; Markowitz, Mann and Bollman, 1929). This and the fact that observed muscle glycogen values are equally low irrespective of the supply of insulin, fit together to offer an explanation, which is in accord with Cori's statements, for the higher liver glycogen obtained in the presence of more insulin. It is probable that the increased muscle glycogen, formed because of the presence of more insulin and a high blood sugar level in the animals which were not depancreatized (table 2), is broken down as fast as formed to lactic acid by the epinephrine. Thus there would be an increase in lactic acid which would be the precursor for more liver glycogen.

It is not suggested that this lactic acid is the only, or even the chief source of liver glycogen in normal dogs injected with epinephrine. The high blood sugar present (Goldblatt, 1929; Corkill, 1930; Bissinger and Lesser, 1926;

Cori, 1925) and inhibition by insulin of the hepatic glycogenolysis (Chaikoff, 1925; Goldblatt, 1929; Cori, Cori and Goltz, 1932; Cori, 1931) which epinephrine brings about (Markowitz, 1925; Sahyun and Luck, 1929; Eadie 1929; Cori, Cori and Buchwald, 1930) are probably contributory causes. What proportion of the process can be assigned to each of these three possible forces cannot be determined here.

In the acute experiments the factor which stimulated the insulin secretion was humoral in nature since the vagus nerves, which supply the islets of Langerhans, were cut in nearly all cases. That humoral factor was most likely the high level of blood sugar. Since the possibility of nervous influence was not ruled out in the experiments on unanesthetized dogs, it is entirely plausible that the smaller sensitivity of the normal animal to injected epinephrine was due partly to nervous effects on the pancreas. This possibility is substantiated by the observation that the difference in sensitivity to epinephrine (judged by blood sugar elevation) is much more striking in the case of the unanesthetized dogs than in those under amytal anesthesia, with cut vagi. This may also be due to amytal.

SUMMARY AND CONCLUSIONS

1. Epinephrine is more effective in raising the blood sugar of a depancreatized dog given insulin in adequate amounts for normal purposes than it is in a normal dog.

Epinephrine administration is followed after several hours by high liver glycogen and low muscle glycogen in normal dogs, and by low glycogen values of both liver and muscle in depancreatized-insulinized dogs.

2. It is concluded that epinephrine stimulates in some fashion (partly humoral) an increased secretion of insulin in a normal dog.

The high liver glycogen values after epinephrine are due to the extra secretion of insulin, which is also responsible for the slower rise of blood sugar following epinephrine in a normal dog than in one which cannot augment its insulin supply.

The possible mechanism of increased liver glycogen formation is discussed.

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